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(54) **NOVEL METALLOPROTEASE AND DNA CODING FOR THE SAME.**

(57) A novel metalloprotease, a DNA coding for the same, a plasmid having the sequence of the DNA, a host cell having the plasmid, and a monoclonal antibody binding specifically to the protein thereof, being useful in the medical and physiological fields for recognizing the presence of a cancer cell, diagnosing cancer malignancy, and other applications.

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TECHNICAL FIELD

The present invention relates to a novel metalloproteinase useful in applications such as diagnosis of the presence of tumour cells, diagnosis of the degree of tumour malignancy, or other medical or physiological fields.

More specifically, the present invention relates to one type of metalloproteinase expressed specifically in human tumour cells and a DNA sequence encoding therefor; a plasmid having a nucleotide sequence which contains said DNA sequence; a host cell harbouring said plasmid; a method for manufacturing said protein using said host cell; a probe which hybridizes with the aforesaid DNA sequence; a method for detecting DNA or RNA containing the aforesaid sequence using said probe; and monoclonal antibodies which bind specifically to the aforesaid protein.

BACKGROUND

A group of enzymes with different substrate specificity and referred to in general as matrix metalloproteinases (hereinafter referred to as "MMPs") contributes to degradation of the extracellular matrix comprising such complex components as collagen, proteoglycan, elastin, fibronectin, and laminin.

Previously reported MMPs include interstitial collagenase (MMP-1), 72 kDa gelatinase (also known as type IV collagenase or gelatinase A; MMP-2), 92 kDa gelatinase (also known as type IV collagenase or gelatinase B; MMP-9), stromelysin-1 (MMP-3), matrilysin (MMP-7), neutrophil collagenase (MMP-8), stromelysin-2 (MMP-10) and stromelysin-3 (MMP-11).

These MMPs are a family of enzymes whose primary structure has been reported previously. With the exception of MMP-7, the primary structure among the family of reported MMPs comprises essentially an N-terminal propeptide domain, a Zn^+ binding catalytic domain and a C-terminal hemopexin-like domain. In MMP-7 there is no hemopexin-like domain. MMP-2 and MMP-9 contain an additional gelatin-binding domain. In addition, a proline-rich domain highly homologous to a type V collagen $\alpha 2$ chain is inserted in MMP-9 between the Zn^+ binding catalytic domain and the C-terminal hemopexin-like domain.

In highly metastatic tumour cells, there are reports of conspicuous expression of type IV collagenase (MMP-2, MMP-9) which mainly degrade type IV collagen (Cancer Res., 46:1-7, 1986; Biochem. Biophys. Res. Commun., 154:832-838, 1988; Cancer, 71:1368-1383, 1993). Likewise, it has been reported MMP-3 act as an activator of proMMP-9 (J. Biol. Chem., 267:3581-3584, 1992).

The degree of matrix metalloproteinase expression serves as an index to diagnosing the degree of cancer malignancy.

DISCLOSURE OF THE INVENTION

The present inventors discovered a novel matrix metalloproteinase (hereinafter deferred to as "MT-MMP") and performed a structural analysis thereof.

As described hereafter, the present invention offers a novel metalloproteinase protein, DNA having a nucleotide sequence which encodes said protein, a plasmid having said DNA nucleotide sequence, a host cell harbouring said plasmid and monoclonal antibodies which specifically recognize the aforesaid metalloproteinase protein.

1. A protein having the amino acid sequence shown in Sequence Sheet sequence number 1.
2. A DNA having the nucleotide sequence shown in Sequence Sheet sequence number 2 which encodes a protein having the amino acid sequence shown in Sequence Sheet sequence number 1.
3. A plasmid containing a DNA having the nucleotide sequence shown in Sequence Sheet sequence number 2, and expressing the protein shown in Sequence Sheet sequence number 1.
4. A host cell harbouring a plasmid containing a DNA having the nucleotide sequence shown in Sequence Sheet sequence number 2, and expressing the protein shown in Sequence Sheet sequence number 1.
5. Monoclonal antibodies which peculiarly recognize a protein having the amino acid sequence shown in Sequence Sheet sequence number 1.

The present invention is described in detail hereafter.

Using highly conserved sequences (Sequence Sheet sequence numbers 3 and 4) selected from amino acid sequences of the known matrix metalloproteinase (MMP) family, the present inventors designed and synthesized an oligonucleotide primer having the sequences denoted by Sequence Sheet sequence numbers 5 and 6. A PCR was carried out using said oligonucleotide primer and a human placental cDNA library, the PCR products obtained were sequenced, and a 390 bp DNA fragment having a sequence non-

homologous to known MMP was obtained. Using this 390 bp DNA fragment as a probe, the human placenta cDNA library was screened, and a cDNA in the positive phage clone obtained was sequenced. The nucleotide sequence is that denoted by Sequence Sheet sequence number 2. A sequence identical to the nucleotide sequence in Sequence Sheet sequence number 2 did not exist in the Genbank/EMBL DNA database, and DNA having this nucleotide sequence was ascertained to be completely novel.

The nucleotide sequence of the aforesaid cloned cDNA in Sequence Sheet sequence number 2 had a 3' non-coding sequence and open reading frame that potentially encode 582 amino acid. An initiation codon was located at nucleotide number 112, and a stop codon was present at nucleotide number 1858. It was determined that this open reading frame encoded the 582 amino acid sequence in Sequence Sheet sequence number 1, that a deduced signal sequence continued after the initiation codon, and that a hydrophobic domain (Sequence Sheet sequence number 7) specific to a membrane-binding protein of 20 or more linked hydrophobic amino acids was present from C-terminal amino acid number 533 to 562.

When homology between the amino acid sequence of MT-MMP and that of the known MMP family was analyzed, MT-MMP had high homology to the known MMP family, as shown in Figure 2. The sequences best conserved in MT-MMP were active site sequences, as well as sequences proximal to processing site between precursor and mature substance conserved in the MMP family. The fact that MT-MMP has the structural characteristics of a membrane-binding protein, and the presence in MT-MMP of a sequence of linked hydrophobic amino acids (shown in Sequence Sheet sequence number 7) not found in the rest of the MMP family, strongly suggested that MT-MMP, unlike other MMP family, is a membrane-binding MMP.

When MT-MMP expression in various human tissues was studied by Northern Blot analysis with various tissue-derived Poly(A)RNA, high expression was seen in the placenta, lung and kidney (see Figure 3). Likewise, results from Northern Blot analysis performed with RNA extracted from normal and tumour areas of human lung squamous cell carcinoma showed that MT-MMP is expressed peculiarly at tumour sites (see Figure 4).

Finally, immunoprecipitation and immunostain experiments using anti-MT-MMP monoclonal antibodies showed that the MT-MMP pertaining to the present invention is expressed on a cell membrane without secretion of a gene product, and MMP-2 activation induced by the expression of MT-MMP was observed in the cells transfected with MT-MMP gene (Nature, 370:61-65, 1994).

Due to the achievements of the above-discussed research by the present inventors, the present invention offers a novel matrix metalloproteinase protein having the amino acid sequence in Sequence Sheet sequence number 1.

In addition, the present invention offers DNA having the nucleotide sequence in Sequence Sheet sequence number 2, which encodes a protein having the amino acid sequence in Sequence Sheet sequence number 1; a plasmid containing and capable of expressing said DNA; and a host cell harbouring said plasmid. All host cells used in general recombinant DNA technology can be used as the aforementioned host cell, including prokaryotes such as E. coli and Baci lus subtilus; eukaryotes such as yeast, COS cells, CHO cells and 3T3 cells; and insect cells such as Sf21. Expression vectors corresponding to used host cells can be used as the aforementioned plasmid.

Furthermore, the present invention offers mRNA transcribed from DNA having the nucleotide sequence in Sequence Sheet sequence number 2.

The present invention also offers a probe which hybridizes with the aforementioned DNA or RNA and specifically detects said DNA or RNA, and said probe may be one having any part of the nucleotide sequence in Sequence Sheet sequence number 2, provided said probe is labeled by a generally used radioactive isotope or enzyme or the like, hybridizes specifically with said DNA or RNA in general blotting analysis and *in situ* hybridization, and accomplishes detection.

Furthermore, the present invention offers monoclonal antibodies which bind peculiarly with the MT-MMP pertaining to the present invention.

The monoclonal antibodies pertaining to the present invention can be prepared by a well-known method such as the method of Milstein et al. (Nature, 256:495-497, 1975) using human MT-MMP as an antigen. In this method, the antigen may be native human MT-MMP, recombinant human MT-MMP, or a synthetic peptide having a partial amino acid sequence of either.

By means of the present invention, DNA having a nucleotide sequence which encodes a protein with the amino acid sequence of the novel MT-MMP pertaining to the present invention can be cloned, and such DNA and a protein encoded by such DNA can be prepared by a genetic engineering technique. Through the use of a cDNA clone of such a novel MT-MMP, techniques generally used in genetic engineering can be used to clone the aforementioned nucleotide sequence into another vector or host. Based on the aforementioned cDNA nucleotide sequence, DNA appropriately suited to a probe may be designed and prepared. In addition, based on the nucleotide sequence of the MT-MMP pertaining to the present invention,

techniques generally used in genetic engineering can be used to prepare a corresponding protein wherein appropriate mutation have been introduced into the MT-MMP amino acid sequence by substitution, deletion, insertion, displacement or addition of one or more amino acids. All such aforementioned derivatives may also be included in the present invention, provided that common metalloproteinase characteristics are conserved; namely, sequences proximal to processing site between precursor and mature substance, active site sequences and domain structure, and provided that the MT-MMP characteristic of a hydrophobic domain of linked hydrophobic amino acids present near the C terminus is conserved.

Use of the above-discussed various implementations of the present invention offers various technical means applicable to applications pertaining to diagnostic agents or diagnostic methods used for diagnosis of the presence of tumour cells or for diagnosis of the degree of tumour malignancy, as well as applications in other medical or physiological fields.

The present invention is described in detail hereafter by means of Working Examples, but the present invention is not limited by these Working Examples.

WORKING EXAMPLES

Working Example 1 Isolation of novel metalloproteinase (MT-MMP) cDNA

(a) Construction of cDNA Library

Total RNA was extracted from human placenta tissue by a guanidine-cesium chloride method (Biochemistry, 18:5294-5299, 1979) and poly(A)$^+$RNA was purified using an oligo(dT)-cellulose column. Using a purified poly(A)$^+$RNA as a template and an oligo(dT) primer, cDNA was synthesized according to the Gubler-Hoffman method (Gene, 25:263-269, 1983). The ends of the cDNA were converted to blunt end with T_4 DNA polymerase, and EcoR I sites present in the cDNA were methylated by EcoR I methylase. Using T_4 DNA ligase, an EcoR I linker [d(pG-G-A-A-T-T-C-C)] and the cDNA were ligated, and cDNA possessing EcoR I sites at both ends was generated by EcoR I digestion. Using T_4 DNA ligase, this cDNA was cloned into EcoR I site of λgt11. *In vitro* packaging of this cDNA was carried out, for example, using an *in vitro* packaging kit (Amersham), and a cDNA library was thus constructed. A commercial cDNA library such as a human placenta cDNA library (Clontech) can be used as a cDNA library.

(b) Preparation of synthetic oligonucleotide primer

The sequences denoted by Sequence Sheet sequence numbers 3 (P-1) and 4 (P-2) were selected from among amino acid sequences of the known MMP family as highly conserved amino acid sequences in the MMP family, and oligodeoxynucleotide primers corresponding respectively to oligopeptide P-1 and oligopeptide P-2 were designed. Specifically, when amino acids coded by two or more codons were present in an oligopeptide, the sequences were designed as a mixture as shown in Sequence Sheet sequence numbers 5 (primer 1) and 6 (primer 2). Primer 1 and primer 2 were synthesized by a β-cyanoethyl phosphoamidite method using a DNA synthesizer (Applied Biosystems Model 392). Using a NICK column (Pharmacia) equilibrated with 10mM sodium phosphate buffer, pH 6.8 the obtained primer 1 and primer 2 were purified.

(c) Gene amplification by PCR

Using a human placenta-derived cDNA as a template and primers 1 and 2 noted in the above section (b), a PCR (PCR Technology, Stockton Press, pp. 63-67, 1989) was run.

As a result, a 390 bp PCR product was yielded. The obtained PCR product was cloned in an appropriate plasmid, e.g., pUC 119 or pBluescript, and the nucleotide sequence of the PCR product was determined using a fluorescence DNA sequencer (Applied Biosystems, Model 373A) and a Taq dye-primer cycle sequencing kit (Applied Biosystems). Among various PCR products whose nucleotide sequences were determined, PCR product A having no homology to nucleotide sequences of previously reported MMPs was obtained. PCR product A was used as a probe for screening the human placenta cDNA library noted in the foregoing section (a). ^{32}P labeling of the probe was generated using a random primed DNA labeling kit (Boehringer Mannhaim).

(d) Screening of novel MMP gene from cDNA library and DNA sequencing.

Host E. coli Y1090 was transfected with the human placenta cDNA library constructed in the λgt11 cited in the foregoing section (a) and plaques were formed. Specifically, Y1090 was cultured overnight in an L broth containing 0.02% maltose, and bacteria were harvested and suspended in 10mM $MgSO_4$. This cell suspension and a phage solution were mixed, incubated at 37 • C for 15 minutes, and then the phages were adsorbed onto the host bacteria. Soft agar was added thereto, and the material was spread on an L plate (the above-noted operation is hereinafter termed "plating"). The plate was incubated overnight at 42 • C and a plaque was formed, after which a nylon filter (e.g., Hibond-N, Amersham) or a nitrocellulose filter (e.g., HATF, Millipore) was placed onto the plate and left in place for approximately 30 seconds. The filter was gently peeled and immersed in an alkaline denaturant (0.1M NaOH, 1.5M NaCl) for 30 seconds, then immersed in a neutralizing solution (0.5M Tris-HCl buffer, pH 8 containing 1.5M NaCl) for 5 minutes. The filter was then washed with 2x SSPE (0.36M NaCl, 20mM NaH_2PO_4, 2mM EDTA) and dried. The foregoing plaque-to-filter transfer was repeated, and at least two filters were prepared. However, plate contact time for the second and subsequent filters was extended to approximately 2 minutes. Filters were baked 2 hours at 80 • C and DNA was thus fixed. The two filters, at a minimum, prepared from one plate were respectively washed 1 hour at 42 • C in a wash solution (50mM Tris-HCl buffer, pH 8.0 containing 1M NaCl, 1mM EDTA and 0.1% SDS), placed in a hybridization bag, and prehybridization was carried out by 6 to 8 hours immersion at 42 • C in a prehybridization solution [50% formamide, 5x Denhardt's solution (0.2% bovine serum albumin, 0.2% polyvinylpyrolidone), 5x SSPE, 0.1% SDS, 100μg/ml heat-denatured salmon sperm DNA]. Next, the ^{32}P-labeled probe noted in section (c), heat-denatured for 5 minutes at 100 • C, was added to the prehybridization solution, and hybridization was carried out overnight at 42 • C. After hybridization was complete, the filters were washed at room temperature with an excess of 2x SSC solution containing 0.1% SDS. Next, the filters were placed for 15 minutes at 68 • C in 1x SSC solution containing 0.1% SDS. The filters were then dried, layered with X-ray film (Kodak XR), and 1 week autoradiography was then carried out at -70 • C. The X-ray films were developed, replica filters in duplicate produced from one plate were piled up each other, and signals that appeared precisely same place on duplicate filters were marked. Plaques corresponding to marked signals were suspended in SM solution (50mM Tris-HCl buffer, pH 7.5 containing 0.1M NaCl and 10mM $MgSO_4$). These phage suspensions were appropriately diluted and plating was performed, screening similar to that noted above was carried out, and recombinant phages were obtained.

(e) Preparation of recombinant λgt11 DNA

Each cloned phages was plated, incubated for 3 hours at 42 • C, and incubated overnight at 37 • C. Several drops of chloroform was then added to the SM solution and the material was left at room temperature for 30 minutes. The SM solution together with the upper layer of soft agar was then scraped off, and centrifuged. Polyethylene glycol was added to a 10% final concentration in the supernatant, and the material was mixed and left at 4 • C for 1 hour. The material was then centrifuged, the supernatant was discarded, and phage particles were collected. The phage particles were suspended in SM solution and purified by a glycerol gradient ultracentrifugation method (see "Molecular Cloning, a Laboratory Manual", T. Maniastis et al., Cold Spring Harbor: Laboratory Press pp. 2.78, 1989). The phages obtained were suspended in SM solution and treated with DNase I and RNase A. A mixture of 20mM EDTA, 50μg/ml proteinase K, and 0.5% SDS was then added, and the material was incubated at 65 • C for 1 hour. The material was then subjected to phenol extraction and diethylether extraction, and DNA was precipitated by ethanol precipitation. The DNA obtained was washed with 70% ethanol, dried, and dissolved in TE solution (10mM Tris-HCl buffer, pH 8 containing 10mM EDTA).

(f) Sequencing of the insertion fragment

The λgt11 DNA prepared in the above section (e) was digested with EcoR I, an insertion fragment was excised and purified, and cloned into the EcoR I site of a pBluescript (Stratagene) vector. E. coli NM522 XLI-Blue was transformed with this recombinant pBluescript. The F' transformed cells were selected, infected with helper phage VCSM13 (Stratagene), and cultured overnight. The culture was centrifuged and the bacteria were removed, and PEG/NaCl was added to precipitate the phag s. The precipitate was suspended in TE solution, and single-stranded DNA was extracted with phenol and recovered by ethanol precipitation. The single-stranded DNA was sequenced using a fluorescence DNA sequencer (Applied Biosystems, Model 373A) and a Taq dye-primer cycle sequencing kit (Applied Biosystems). The total length

of the sequence determined was 3403 base pairs, and the sequence thereof is denoted by Sequence Sheet sequence number 2. The nucleotide sequence in Sequence Sheet sequence number 2 was searched using the Genbank/EMBL DNA database, but an identical sequence did not exist.

(g) Analysis of Gene Product

Hydrophilic and hydrophobic values of the amino acid sequence denoted by Sequence Sheet sequence number 1, as predicted from the nucleotide sequence denoted by Sequence Sheet sequence number 2, were calculated by the Kyte-Doolittle method (J. Mol. Biol., 157:105-132, 1982), and the hydrophilic and hydrophobic distribution plot shown in Figure 1 was determined. A hydrophobic domain comprising a sequence of 20 or more linked hydrophobic amino acids peculiar to a membrane binding protein was present from position 533 to position 562 of the C-terminal region of Sequence Sheet sequence number 1, and the sequence thereof is shown in Sequence Sheet sequence number 7. Such a sequence of linked hydrophobic amino acids does not exist in previously known MMPs.

When the homology of the amino acid sequence in Sequence Sheet sequence number 1 was compared to reported MMPs amino acid sequences, the amino acid sequence in Sequence Sheet sequence number 1 showed homology with the MMP family. Specifically, processing site between precursor and active enzyme and active site conserved to an extremely high degree among MMP family were each highly conserved in MT-MMP as well (Sequence Sheet sequence number 1, amino acids numbers 88-97 and 112-222).

Working Example 2 Gene Expression

(a) Expression in Tissues

Using ^{32}P-labeled PCR product A noted in Working Example 1, section (c) as a probe, hybridization was performed with poly(A)$^+$ RNA blotted membrane, human multiple tissue Northern Blots (Clontech), which contains poly(A)$^+$ RNA from human heart, brain, placenta, lung, liver, skeletal muscle, kidney and pancreas. Human multiple tissue Northern Blot filters wetted with 3x SSC (0.45M NaCl, 0.045M trisodium citrate•2$H_2$0, pH 7.0) were prehybridized for 2 to 3 hours in a prehybridization solution (0.75M NaCl, 2.5mM EDTA, 0.5x Denhardt's solution, 50% formamide and 20mM Tris-HCl buffer, pH 7.5 containing 1% SDS) with gentle agitation. Next, a heat-denatured probe was added to the hybridization solution (10% sodium dextran and 50µg/ml denatured salmon sperm DNA-containing prehybridization solution), the prehybridization solution was replaced, and hybridization was performed overnight at 43•C. After hybridization was complete, the filters were washed with 2x SSC containing 0.1% SDS. Next, the filters were placed for 15 minutes at 68•C in 1x SSC containing 0.1% SDS. The filters were then dried, layered with X-ray film (Kodak XR), and 1 week autoradiography was then carried out at -70•C. The size of the MT-MMP gene transcripts was 4.8 kb in each tissue. When the developed X-ray films were traced by a densitometer and signal intensity was measured, among the investigated tissues, MT-MMP genes were found to be highly expressed in the lung, placenta and kidney.

(b) Expression in Tumour Tissues

Normal and tumour tissues were taken from samples of two squamous cell carcinomas human lung, respectively, and total RNA was extracted by a guanidine-cesium chloride method. 10µg of each said RNA was applied to 1% agarose electrophoresis and then transferred onto a nylon membrane. Hybridization was then carried out with the ^{32}P-labeled probe noted in Working Example 1, section (c). Hybridization and autoradiography tracing were performed as described in the foregoing section (a). In each human lung squamous cell carcinoma, significantly higher expression were seen in tumour tissue (see Figure 4 T) than in normal tissue (see Figure 4 N).

Working Example 3 Preparation of Monoclonal Antibodies

(a) Preparation of Polypeptides as Antigen

From the MT-MMP amino acid sequence denoted by Sequence Sheet sequence number 1, sequences denoted by Sequence Sheet sequence numbers 8, 9 and 10 (sequence of Sequence Sheet sequence number 1 amino acid numbers 160-173, 320-333, and 498-512, respectively; hereinafter termed polypeptide

A, polypeptide B and polypeptide C, respectively) were selected as specific sequences having low homology to other members of MMP family. These polypeptides were synthesized by Fmoc-BOP method using a peptide synthesizer (MilliGen/Biosearch, Peptide Synthesizer 9600), and cysteine was introduced at the N-terminus. Each synthesized peptide was purified by high speed liquid chromatography.

(b) Preparation of Each Polypeptides and Keyhole Limpet Hemocyanin Complexes

2 mg of keyhole limpet hemocyanin (KLH) dissolved in 1 ml of 0.1M phosphate buffer, pH 7.5 and 1.85 mg N-(ε-maleimidocaproyloxy)succinimide dissolved in 200 µl dimethylformamide were mixed and incubated at 30 • C for 30 minutes. Next, the above-noted mixture was applied to gel filtration by PD-10 (Pharmacia) equilibrated with 0.1M phosphate buffer, pH 7.0. KLH-bound maleimide was collected and concentrated to less than 1.5 ml. Each polypeptide synthesized in the foregoing section (a) was respectively dissolved in 1 ml of 0.1M phosphate buffer, pH 7.0 and mixed with KLH-bound maleimide at a molar ratio representing a factor of 50. This material was then incubated 20 hours at 4 • C, and KLH-polypeptide complexes were thus prepared.

(c) Preparation of Antibody-producing Cells

As an initial immunization, eight-week-old Balb/c female mice were given an intraperitoneal administration of 250 µg of a complex of KLH and, respectively, polypeptide A, polypeptide B or polypeptide C prepared in the above section (b), in Freund's complete adjuvant. After 18 days, the respectively immunized mice were boosted intraperitoneally with 200 µg of the respective complexes dissolved in 0.1M phosphate buffer, pH 7.5. After 32 days, a final immunization of 100 µg of each complex was administered intravenously as the booster immunization. Three days thereafter, splencytes were extirpated and splencyte suspensions were prepared.

(d) Cell Fusion

Fusion with 8-azaguanine-resistant myeloma cell SP2 (SP2/O-Ag14) was performed according to a modifying method of Oi et al (see Selected Methods in Cellular Immunology, Mishell, B.B. and Shiigi, S. M., ed., W.H. Freeman and Company pp. 351-372, 1980). Fusion of myeloma cell SP2 with karyo-splencytes from mice immunized with the polypeptide A-KLH complex is discussed in details, hereafter.

Through the following procedures, karyo-splencytes prepared in the foregoing section (c) (cell viability 100%) were fused in a 5:1 ratio with myeloma cells (cell viability 100%). A polypeptide A-immunized splencyte suspension and myeloma cells were separately washed in RPMI 1640 medium. The material was then suspended in the same medium, and $3x10^8$ cells of karyo-splencytes and $6x10^7$ cells of myeloma cells were mixed for fusion. The cells were then precipitated by centrifugation, and all the supernatant was completely discarded by suction. 2.0 ml of PEG 4000 solution (RPMI 1640 medium containing 50% [w/v] polyethylene glycol 4000) prewarmed at 37 • C was added dropwise to the precipitated cells over 1 minute, 1 minute stirring was performed, and the cells were resuspended and dispersed. Next, 2.0 ml of RPMI 1640 medium prewarmed at 37 • C was added in a dropwise fashion over 1 minute. After repeating the same operation once more, 14 ml of RPMI 1640 medium was added dropwise over 2 to 3 minutes under constant stirring, and the cells were dispersed. The dispersion was centrifuged and the supernatant was completely discarded by suction. Next, 30 ml of NS-1 medium (RPMI 1640 medium containing filter-sterilized 15% [w/v] fetal calf serum [JRH Biosciences]) prewarmed at 37 • C was rapidly added to the precipitated cells, and the large cell clumps were carefully dispersed by pipetting. The dispersion was then diluted by adding 30 ml of NS-1 medium, and $6.0x10^5$ cells/0.1 ml/well was added to a polystyrene 96-microwell plate. The above-noted cell-filled microwells were cultured in 7% carbonic acid gas/93% atmospheric air at 37 • C and 100% humidity.

In the case of splencytes derived from mice immunized with the polypeptide B-KLH complex, $6.4x10^8$ cells of splencytes and $1.28x10^8$ cells of myeloma cells were mixed, and respectively, 4.3 ml, 38.7 ml and 129 ml of the above-used PEG 4000 solution, RPMI 1640 medium and NS-1 medium were used. In the case of splencytes derived from mice immunized with the polypeptide C-KLH complex, $6.8x10^8$ cells of splencytes and $1.36x10^8$ cells of myeloma cells were mixed, and 4.5 ml, 40.5 ml and 135 ml of respectively PEG 4000 solution, RPMI 1640 medium and NS-1 medium were used.

(e) Selective Amplification of Hybridomas by Selective Culture Medium

On the day following the start of culturing mentioned in the above section (d) (Day 1), 2 drops (approx. 0.1 ml) HAT culture medium (100 μM hypoxanthine, 0.4 μM aminopterin and 16 μM thymidine added to NS-1 culture medium) were added to the cells with a Pasteur pipette. On Days 2, 3, 5 and 8, half of each culture medium (approx. 0.1 ml) was replaced with fresh HAT medium, and on Day 11, half of each culture medium was replaced with fresh HT culture medium (HAT culture medium not containing aminopterin). On Day 14, for all the wells in which hybridoma growth was observed to the naked eye, positive wells were investigated by enzyme-linked immunoadsorbent assay (ELISA). Specifically, the polystyrene 96-well plate was respectively coated with polypeptides A, B and C serving as antigens, washed using PBS for washing (containing 0.05% Tween 20), and unadsorbed peptides were thus removed. In addition, the uncoated portion of each well was blocked with 1% BSA. 0.1 ml of supernatant from wells in which hybridoma growth was confirmed was added to each polypeptide-coated well, and the plate was stood at room temperature for approximately 1 hour.

Horseradish peroxidase-labeled goat anti-mouse immunoglobulin was added as a secondary antibody, and the plate was again stood at room temperature for approximately another 1 hour. A substrate of hydrogen peroxide and o-phenylenediamine was added, and the degree of color development was measured as absorbance at 492 nm using a microplate light absorbency measuring device (MRP-A4, Tosoh).

(f) Hybridoma Cloning

Hybridomas in wells positive with respect to individual antigen peptides, as obtained in the foregoing section (e), were monocloned according to the limiting dilution method. Specifically, hybridomas were diluted to 5, 1 and 0.5 per well and were respectively added to 36, 36 and 24 wells of a 96 microwells. On Day 5 and Day 12, approximately 0.1 ml NS-1 medium was added to each well. Approximately 2 weeks after cloning began, the ELISA noted in section (e) was performed for groups in which sufficient hybridoma growth was visually confirmed and 50% or more wells were negative for colony formation. If all tested wells were not positive, 4 to 6 antibody-positive wells in which the number of colonies was 1 were selected, and recloning was performed. Finally, as shown in Table 1 and Table 2, 12, 20 and 9 hybridomas were obtained which produced monoclonal antibodies against polypeptide A, polypeptide B or polypeptide C, respectively.

(g) Hybridoma Culturing and Monoclonal Antibody Purification

Each obtained hybridoma was cultured in NS-1 medium and a 10 to 100 μg/ml concentration of monoclonal antibody was successfully obtained from the supernatant thereof. In addition, BALB/c mice given an one week prior intraperitoneal administration of pristane were given a similar intraperitoneal administration of $1x10^7$ cells of obtained hybridomas, and after 1 to 2 weeks, abdominal fluid containing 4 to 7 mg/ml of monoclonal antibody was successfully obtained. The abdominal fluid obtained was salted out by 40% saturated ammonium sulfate, and IgG class antibodies were adsorbed to Protein A Affigel (Bio-Rad) and purified by elution with a 0.1M citric acid buffer, pH 5.

(h) Determination of Monoclonal Antibody Class and Subclass

In accordance with the above-discussed ELISA, the supernatant of monoclones obtained in section (f) were added to microtitration plates respectively coated with polypeptide A, polypeptide B or polypeptide C. After washing with PBS, isotype-specific rabbit anti-mouse IgG antibodies (Zymed Lab.) were added. After washing with PBS, horseradish peroxidase-labeled goat anti-rabbit IgG (H+L) was added, and class and subclass were determined using hydrogen peroxide and 2.2'-azino-di(3-ethylbenzthiazolinic acid) as a substrate.

(i) Specificity of Anti-MT-MMP Monoclonal Antibodies

The cross-reactivity of five varieties of anti-MT-MMP monoclonal antibodies (monoclone numbers 113-5B7, 113-15E7, 114-1F1, 114-2F2 and 118-3B1) exhibiting a positive reaction against a human MT-MMP peptide was determined by the ELISA noted in the foregoing section (e), using as respective antigens: proMMP-1 (Clin. Chim. Acta, 219:1-14, 1993), proMMP-2 (Clin. Chim. Acta, 221:91-103, 1993) and proMMP-3 (Clin. Chim. Acta, 211:59-72, 1992) respectively purified from the supernatant of nomal human skin

fibroblast (NB1RGB) culture; proMMP-7 purified from the supernatant of human rectal carcinoma cell (CaR-1) culture (Cancer Res., 50:7758-7764, 1990), proMMP-8 purified from human neutrophils (Biol. Chem. Hoppe-Seyler, 371 supp:295-304, 1990) and proMMP-9 purified from the supernatant of human fibrosarcoma cells (HT1080) culture (J. Biol. Chem., 267: 21712-21719, 1992).

Specifically, using a polystyrene 96-well plate, each well was coated by adding 50 ng/well of purified MMP-1, MMP-2, MMP-3, MMP-7, MMP-8 and MMP-9, respectively. Washing was performed with PBS for washing and non-adsorbed antigen was removed, and the uncoated portion of each well was blocked with PBS containing 3% skim milk. 1 μg/well of each MT-MMP monoclonal antibody was respectively added to each well and stood at room temperature for approximately 1 hour. After washing plate, peroxidase-labeled goat anti-mouse immunoglobulin was added as a secondary antibody, and the plate was again stood at room temperature for approximately 1 hour. A substrate of hydrogen peroxide and o-phenylene diamine was added, and the degree of color development was measured absorbance at 492 nm using a microplate light absorbency measuring device (MRP-A4, Tosoh).

In results, as shown in Table 3, each anti-MT-MMP monoclonal antibody showed no reactivity against purified MMPs other than the MT-MMP supplied for testing.

TABLE 1

Polypeptide	Monoclone No.	Subclass/Chain
A	114-1F2	γ1/κ
	114-2F2	γ1/κ
	114-3H7	γ1/κ
	114-5E4	γ1/κ
	114-6G6	γ1/κ
	114-8D10	γ1/κ
	114-9H3	μ/κ
	114-15E8	γ1/κ
	114-16C11	γ1/κ
	114-18E4	γ1/κ
	114-19F11	γ1/κ
	114-20H5	μ/κ
B	113-1E3	γ3/κ
	113-2E9	γ3/κ
	113-3F6	γ2b/κ
	113-4H7	γ3/κ
	113-5B7	γ3/κ
	113-7C6	γ2b/κ
	113-9G9	γ3/κ
	113-10F2	γ3/κ
	113-13G11	γ3/κ
	113-15E7	γ3/κ
	113-16H8	γ3/κ
	113-17G12	μ/κ
	113-19A10	μ/κ
	113-20G11	γ3/κ
	113-21H3	γ1/κ
	113-26D3	μ/κ
	113-44C1	γ1/κ
	113-46B7	γ1/κ
	113-53G5	μ/κ
	113-63E8	γ1/κ

TABLE 2

Polypeptide	Monoclone No.	Subclass/Chain
C	118-3B1	γ2b/κ
	118-6F3	γ2b/κ
	118-8D11	γ1/κ
	118-9B11	γ1/κ
	118-13D11	α/κ
	118-18C12	γ1/κ
	118-20A3	γ2b/κ
	118-25C3	γ1/κ
	118-26F5	γ3/κ

TABLE 3

Monoclone No.	Cross reactivity					
	MMP-1	MMP-2	MMP-3	MMP-7	MMP-8	MMP-9
113-5B7	-	-	-	-	-	-
113-15E7	-	-	-	-	-	-
114-1F2	-	-	-	-	-	-
114-2F2	-	-	-	-	-	-
118-3B1	-	-	-	-	-	-
- :No reaction						

Working Example 4 Expression and Identification of Gene Product

By means of EcoR I cleavage, an insertion fragment was excised from the recombinant pBluescript containing a cloned MT-MMP gene, constructed in section (f) of Working Example 1. Cloning was then carried out at an EcoR I site of the eukaryotic expression vector pSG5 (Stratagene). Then, human fibrosarcoma cells (HT1080) were transfected with said recombinant pSG5 by a calcium phosphate method. Specifically, 20 μg of recombinant pSG5 and 62 μl of 2M $CaCl_2$ was added to distilled water, and 2x HBSP solution (50mM HEPES buffer, pH 7.1 containing 1.5mM Na_2HPO_4, 10mM KCl, 280mM NaCl and 12mM glucose) was added to the bottom of the tube to form a total volume of 1 ml. This material was mixed, stood at room temperature for approximately 30 minutes, and thorough precipitate formation was carried out. The precipitate was dispersed by pipetting, added dropwise to HT1080 cells and incubated for approximately 4 hours in a CO_2 incubator. Next, the culture medium was removed, a 15% glycerol solution was added and treated for 1 to 3 hours, the glycerol was discarded by suction, washed with PBS and fresh culture medium containing ^{35}S-methionine was added. Culturing was continued, and cellular proteins were labeled by ^{35}S. Incidentally, expression of MT-MMP genes in HT1080 cells cannot be detected by Northern Blot analysis.

The cells were incubated for 1 hour at 4°C in a lysing buffer solution (0.01M Tris-HCl buffer, pH 8 containing 1% Triton X-100, 1% bovine hemoglobin, 1mM iodoacetamide, 0.2U trypsin inhibitor, 1mM PMSF and 0.14M NaCl). The cell lysate was centrifuged and the supernatant was recovered. Sepharose-4B (Pharmacia) coupled with a monoclonal antibody obtained in Working Example 3 was added to the supernatant, the material was incubated at 4°C for 2 hours with agitation, and immunoprecipitation was carried out. Monoclonal antibodies against polypeptide A used in immunoprecipitation were two of the 12 obtained in Working Example 3 which had low non-specific reactivity (monoclone numbers 114-1F2 and 114-2F2 [Assignment No. FERM BP-4743]). Next, Sepharose 4B coupled with monoclonal antibodies subjected to immunoprecipitation were precipitated by centrifugation, washed three times with a washing solution (0.01M Tris-HCl buffer, pH 8 containing 1% Triton X-100, 1% bovine hemoglobin and 0.14M NaCl), and lastly, washed with 0.05M Tris-HCl buffer, pH 6.8. A sample buffer for SDS polyacrylamide electrophoresis was added to washed Sepharose-4B coupled with a monoclonal antibody, boiled 5 minutes at

100 • C, and SDS polyacrylamide electrophoresis was carried out. The electrophoresed gel was layered with X-ray film (Kodak XR), 1 week autoradiography was then carried out at -70 • C, and the developed X-ray films were traced by a densitometer to measure signal intensity. With each of the anti-MT-MMP monoclonal antibodies used (monoclone numbers 114-1F2 and 114-2F2), the immunoprecipitate contained a 63 kDa protein. In cells transfected with a pSG5 vector alone not containing an MT-MMP gene as a control, anti-MT-MMP monoclonal antibodies (monoclone numbers 114-1F2 and 114-2F2) did not precipitate a 63 kDa protein. The 63 kDa molecular weight of the protein detected by immunoprecipitation nearly matched a molecular weight of 65.78 kDa calculated from the amino acid sequence denoted by Sequence Sheet sequence number 1. In addition, a variant MT-MMP expression plasmid was constructed in which amino acids from position 13 to position 101 were deleted from the amino acid sequence denoted by Sequence Sheet sequence number 1, HT1080 cells was transfected with said variant as stated above, and immunoprecipitation was carried out. With HT1080 cells to which the variant MT-MMP gene was introduced, a 63 kDa protein was not detected, and a 55 kDa protein was detected. This molecular weight matched a molecular weight predicted from the introduced deletion.

EXPERIMENTAL EXAMPLE

(a) Activation of proMMP-2 by MT-MMP Expression

Recombinant pSG5 carrying a cloned MT-MMP gene, constructed in Working Example 4, and a pSG5 vector alone, serving as a control, similarly transfected into HT1080 cells by the calcium phosphate method mentioned in Working Example 4, or into mouse embryonic fibroblasts NIH3T3. However, a regular fresh culture medium was used in lieu of the fresh culture medium containing ^{35}S-methionine. Both the HT1080 cells and the NIH3T3 cells secreted proMMP-2 and proMMP-9 (corresponding respectively to the 66 kDa and 97.4 kDa bands in Figure 6), and in cells transfected with an MT-MMP gene, MT-MMP expression was confirmed by immunoprecipitation experiments (See Working Example 4).

The transfectants obtained were cultured for 24 hours in a serum free medium and the recovered culture supernatant was supplied for zymography. The culture supernatant was mixed with an SDS polyacrylamide electrophoresis buffer (non-reducing condition) and left at 4 • C overnight. Electrophoresis was then performed at 4 • C, with a 20 mA current, using a 10% polyacrylamide gel containing 1 mg/ml casein. After electrophoresis, the gel was washed with a gelatinase-buffer (Tris-HCl buffer, pH 7.6 containing 5mM $CaCl_2$ and 1 μM $ZnSO_4$) containing 2.5% Triton X-100 with gentle agitation for 15 minutes, and this operation was repeated twice. Next, the gel was immersed in a gelatinase-buffer containing 1% Triton X-100 and stood at 37 • C overnight. The buffer was discarded and the gel was stained for 1 hour with 0.02% Coomassie Brilliant Blue-R (dissolved in 50% methanol/10% acetic acid) and destained by immersion in a destaining solution (5% methanol, 7.5% acetic acid).

As shown in Figure 6, MT-MMP gene-transfected HT1080 cells produced new 64 kDa and 62 kDa bands, confirming proMMP-2 activation. This active-form MMP-2 exhibited the same molecular weight as an active-form MMP-2 molecule induced by treatment of cells with 100 μg/ml of concanavalin A and reacted specifically against anti-MMP-2 monoclonal antibodies. This activation was not observed in a control transfected with a vector alone. Likewise, proMMP-9 showed no change in molecular weight and no activation similar to that observed in control cells. Such activation of proMMP-2 depending on MT-MMP expression was also observed in MT-MMP gene-transfected NIH3T3 cells.

(b) Activation of ProMMP-2 by MT-MMP Expression Cell Membrane Fraction

In a manner similar to that noted in the above section (a), African green monkey kidney-derived COS-1 cells were transfected with recombinant pSG5 containing cloned MT-MMP gene, or with control pSG5 vector alone by a calcium phosphate method. A cell membrane fraction was then prepared from the obtained transfectant according to the method of Strongin et al. (J. Biol. Chem., 268:14033-14039, 1993).

The transfectant was washed with PBS, and cells were harvested by centrifugation and suspended in a 25mM Tris-HCl buffer, pH 7.4 containing 8.5% sucrose, 50mM NaCl, 10mM N-ethylmaleimide, 10 μg/ml aprotinin, 1 μg/ml pepstatin A, 1 μg/ml leupeptin and 1mM phenylmethylsulfonyl fluoride. The cell suspension was homogenized in a Dounce homogeniz r, and the homogenate was centrifuged (3000x g, 10 min., 4 • C). The r sulting supernatant was ultracentrifuged (100,000x g, 2 hours) and the precipitate was suspended in a 25mM Tris-HCl buffer, pH 7.4 containing 50mM NaCl, 10mM N-ethylmaleimide, 10 μg/ml aprotinin, 1 μg/ml pepstatin A, 1 μg/ml leupeptin and 1mM phenylmethylsulfonyl fluoride. This suspension was fractionated by discontinuous sucrose density gradient centrifugation (20, 30, 50, 60% sucrose

solutions; 100,000x g; 2 hours; 4 • C), and bands of cell membrane fractions appeared were recovered. These fractions were precipitated again by ultracentrifugation (100,000x g, 2 hours), suspended in 25mM HEPES/KOH buffer, pH 7.5 containing 0.1mM $CaCl_2$ and 0.25% Triton X-100, and adjusted to a final protein concentration of 1-2 mg/ml. This suspension was ultracentrifuged (100,000x g, 1.5 hours, 4 • C) to remove insoluble residue, and the supernatant obtained was taken as a cell membrane fraction.

Cell membrane fractions (protein content 20 μg) respectively prepared from untreated COS-1 cells or from COS-1 cells transfected with pSG5 vector alone or pSG5 vector with an MT-MMP gene were incubated with HT1080 cell culture supernatant at 37 • C for 2 hours. Using these samples, the zymography noted in the above section (a) was performed.

In the results, new 64 kDa and 62 kDa bands appeared and the activation of proMMP-2 present in HT1080 cell culture supernatant was observed only when cell membrane fractions derived from MT-MMP gene-transfected COS-1 cells were used (see Figure 7), and the activation of proMMP-2 was inhibited by the addition of recombinant (r) human TIMP-2. These results exhibited the activation of proMMP-2 by MT-MMP expressed on a cell membrane.

(c) Stimulation of cellular invasion *in vitro* due to MT-MMP expression

Invasion of cells was assayed by modified Boyden Chamber method (Cancer Res., 47:3239-3245, 1987), and operations were carried out in accordance with the manufucture's instructions for a Biocoat Matrigel Invasion Chamber (Becton Dickinson).

In a manner similar to that noted in the foregoing section (a), HT1080 cells or NIH3T3 cells were transfected with recombinant pSG5 carrying a cloned MT-MMP gene, or a control pSG5 vector alone, by a calcium phosphate method, and each of these host cells secreted proMMP-2. The resulting transfectants were then suspended in DMEM medium containing 0.1% BSA, and $2x10^5$ cells were seeded onto an uncoated filter (pore size 8 μm) or a preswelled Matrigel Coat filter in a Biocoat Matrigel Invasion Chambers. After 24 hours incubation in a CO_2 incubator at 37 • C, the filters were fixed by 10 seconds immersion in methanol. The filters were then stained by hematoxylin for 3 minutes, washed, and stained by eosin for 10 seconds, and the number of cells invaded the bottom surface of the filters were counted under a light microscope (at a magnification of x 400).

In the MT-MMP gene-transfected HT1080 cells and NIH3T3 cells, more than twice as many invading cells were seen compared to cells transfected with the control vector alone (See Figure 8 Matrigel). Specifically, MT-MMP expression was seen to stimulate cellular invasion. Furthermore, the addition of 10 μg/ml of r-human TIMP-2 to this assay system clearly suppressed cellular invasion (see Figure 8 Matrigel + r-human TIMP-2).

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 shows hydrophilic and hydrophobic distribution diagrams for the amino acid sequence of MT-MMP, according to the Kyte-Doolittle method.

Figures 2A, 2B, 2C, 2D, 2E, 2F, 2G and 2H are figures comparing sequential homology between the amino acid sequences of MT-MMP and those of the known MMP family (MMP-1, MMP-2, MMP-3, MMP-7, MMP-8, MMP-9, MMP-10 and MMP-11). Letters in each figure indicate respective amino acids; A corresponding to Ala, C to Cys, D to Asp, E to Glu, F to Phe, G to Gly, H to His, I to Ile, K to Lys, L to Leu, M to Met, N to Asn, P to Pro, Q to Gln, R to Arg, S to Ser, T to Thr, V to Val, W to Trp and Y to Tyr. Figures 2A through 2H are an integral unit and comprise a single figure.

Figure 3 shows a relative expression of MT-MMP mRNA in various human tissues, according to Northern blot analysis.

Figure 4 shows a relative expression of MT-MMP mRNA in a normal tissue and a tumour tissue of two samples of human lung squamous cell carcinoma, according to Northern blot analysis.

Figure 5 shows results for detection, by immunoprecipitation, of MT-MMP proteins expressed in HT1080 cells transfected with MT-MMP cDNA. The figure shows a scan by a densitometer, and the darkened areas indicate the location of MT-MMP immunoprecipitated by anti-MT-MMP monoclonal antibody.

Figure 6 shows an activation of proMMP-2 by expression of MT-MMP, according to zymography of culture supernatant from HT1080 and NIH3T3 cells transfected with MT-MMP cDNA.

Figure 7 shows an activation of proMMP-2 by a cell membrane fraction of COS-1 cells transfected with MT-MMP cDNA, according to zymography.

Figure 8 shows a stimulation of the cellular invasion by expression of MT-MMP, according to a partially modified Boyden chamber method.

[Sequence Sheet 1]

Sequence No.: 1

Length of sequence: 582

Type of sequence: Amino acid

Topology: Linear

Class of sequence: Protein

Sequence

```
Met Ser Pro Ala Pro Arg Pro Ser Arg Cys Leu Leu Leu Pro Leu
 1               5                  10                  15
Leu Thr Leu Gly Thr Ala Leu Ala Ser Leu Gly Ser Ala Gln Ser
            20                  25                  30
Ser Ser Phe Ser Pro Glu Ala Trp Leu Gln Gln Tyr Gly Tyr Leu
            35                  40                  45
Pro Pro Gly Asp Leu Arg Thr His Thr Gln Arg Ser Pro Gln Ser
            50                  55                  60
Leu Ser Ala Ala Ile Ala Ala Met Gln Lys Phe Tyr Gly Leu Gln
            65                  70                  75
Val Thr Gly Lys Ala Asp Ala Asp Thr Met Lys Ala Met Arg Arg
            80                  85                  90
Pro Arg Cys Gly Val Pro Asp Lys Phe Gly Ala Glu Ile Lys Ala
            95                  100                 105
Asn Val Arg Arg Lys Arg Tyr Ala Ile Gln Gly Leu Lys Trp Gln
            110                 115                 120
His Asn Glu Ile Thr Phe Cys Ile Gln Asn Tyr Thr Pro Lys Val
            125                 130                 135
Gly Glu Tyr Ala Thr Tyr Glu Ala Ile Arg Lys Ala Phe Arg Val
            140                 145                 150
Trp Glu Ser Ala Thr Pro Leu Arg Phe Arg Glu Val Pr  Tyr Ala
            155                 160                 165
```

[Sequence Sheet 2]

Sequence No.: 1 (continued)

Tyr Ile Arg Glu Gly His Glu Lys Gln Ala Asp Ile Met Ile Phe
170 175 180

Phe Ala Glu Gly Phe His Gly Asp Arg Thr Ala Phe Asp Gly Glu
185 190 195

Gly Gly Phe Leu Ala His Ala Tyr Phe Pro Gly Pro Asn Ile Gly
200 205 210

Gly Asp Thr His Phe Asp Ser Ala Glu Pro Trp Thr Val Arg Asn
215 220 225

Glu Asp Leu Asn Gly Asn Asp Ile Phe Leu Val Ala Val His Glu
230 235 240

Leu Gly His Ala Leu Gly Leu Glu His Ser Ser Asp Pro Ser Ala
245 250 255

Ile Met Ala Pro Phe Tyr Gln Trp Met Asp Thr Glu Lys Phe Val
260 265 270

Leu Pro His Tyr Asp Pro Arg Gly Ile Gln Gln Leu Tyr Gly Gly
275 280 285

Lys Gln Gly Ser Pro Pro Arg Cys Pro Leu Asn Pro Gly Leu Pro
290 295 300

Pro Gly Leu Leu Phe Leu Ile Asn Pro Lys Asn Pro Thr Tyr Gly
305 310 315

Pro Asn Ile Cys Asp Gly Asn Phe Asp Thr Val Ala Met Leu Arg
320 325 330

Gly Glu Met Phe Asp Phe Lys Lys Arg Trp Phe Trp Arg Val Arg
335 340 345

Asn Asn Gln Val Met Asp Gly Tyr Pro Met Pro Ile Gly Gln Phe
350 355 360

Trp Arg Gly Leu Pro Ala Ser Ile Asn Thr Ala Tyr Glu Arg Lys
365 370 375

[Sequence Sheet 3]

Sequence No.: 1 (continued)

```
Asp Gly Lys Phe Val Phe Phe Lys Gly Asp Lys His Trp Val Phe
                380                 385                 390
Asp Glu Ala Ser Leu Glu Pro Gly Tyr Pro Lys His Ile Lys Glu
                395                 400                 405
Leu Gly Arg Gly Leu Pro Thr Asp Lys Ile Asp Ala Ala Leu Phe
                410                 415                 420
Trp Met Pro Asn Gly Lys Thr Tyr Phe Phe Arg Gly Asn Lys Tyr
                425                 430                 435
Tyr Arg Phe Asn Glu Glu Leu Arg Ala Val Asp Ser Glu Tyr Pro
                440                 445                 450
Lys Asn Ile Lys Val Trp Glu Gly Ile Pro Glu Ser Pro Arg Gly
                455                 460                 465
Ser Phe Met Gly Ser Asp Glu Val Phe Thr Tyr Phe Tyr Lys Gly
                470                 475                 480
Asn Lys Tyr Trp Lys Phe Asn Asn Gln Lys Leu Lys Val Glu Pro
                485                 490                 495
Gly Tyr Pro Lys Ser Ala Leu Arg Asp Trp Met Gly Cys Pro Ser
                500                 505                 510
Gly Gly Arg Pro Asp Glu Gly Thr Glu Glu Glu Thr Glu Val Ile
                515                 520                 525
Ile Ile Glu Val Asp Glu Glu Gly Gly Gly Ala Val Ser Ala Ala
                530                 535                 540
Ala Val Val Leu Pro Val Leu Leu Leu Leu Leu Val Leu Ala Val
                545                 550                 555
Gly Leu Ala Val Phe Phe Phe Arg Arg His Gly Thr Pro Arg Arg
                560                 565                 570
Leu Leu Tyr Cys Gln Arg Ser Leu Leu Asp Lys Val
                575                 580
```

[Sequence Sheet 4]

Sequence No.: 2

Length of sequence: 3403

Type of sequence: Nucleic acid

Number of chain: Double strand

Topology: Linear

Class of sequence: cDNA to mRNA

Origin:

Species: Human

Tissue: Placenta

[Sequence Sheet 5]

Sequence No.: 2 (continued)

```
AGTTCAGTGCCTACC GAAGACAAAGGCGCC CCGAGGGAGTGGCGG TGCGACCCCAGGGCG      60

TGGGCCCGGCCGCGG AGCCACACTGCCCGG CTGACCCGGTGGTCT CGGACCATGTCTCCC     120
                                                    MetSerPro
                                                    1

GCCCCAAGACCCTCC CGTTGTCTCCTGCTC CCCCTGCTCACGCTC GGCACCGCGCTCGCC     180
AlaProArgProSer ArgCysLeuLeuLeu ProLeuLeuThrLeu GlyThrAlaLeuAla
5               10              15              20

TCCCTCGGCTCGGCC CAAAGCAGCAGCTTC AGCCCCGAAGCCTGG CTACAGCAATATGGC     240
SerLeuGlySerAla GlnSerSerSerPhe SerProGluAlaTrp LeuGlnGlnTyrGly
25              30              35              40

TACCTGCCTCCCGGG GACCTACGTACCCAC ACACAGCGCTCACCC CAGTCACTCTCAGCG     300
TyrLeuProProGly AspLeuArgThrHis ThrGlnArgSerPro GlnSerLeuSerAla
45              50              55              60

GCCATCGCTGCCATG CAGAAGTTTTACGGC TTGCAAGTAACAGGC AAAGCTGATGCAGAC     360
AlaIleAlaAlaMet GlnLysPheTyrGly LeuGlnValThrGly LysAlaAspAlaAsp
65              70              75              80

ACCATGAAGGCCATG AGGCGCCCCCGATGT GGTGTTCCAGACAAG TTTGGGGCTGAGATC     420
ThrMetLysAlaMet ArgArgProArgCys GlyValProAspLys PheGlyAlaGluIle
85              90              95              100

AAGGCCAATGTTCGA AGGAAGCGCTACGCC ATCCAGGGTCTCAAA TGGCAACATAATGAA     480
LysAlaAsnValArg ArgLysArgTyrAla IleGlnGlyLeuLys TrpGlnHisAsnGlu
105             110             115             120

ATTACTTTCTGCATC CAGAATTACACCCCC AAGGTGGGCGAGTAT GCCACATACGAGGCC     540
IleThrPheCysIle GlnAsnTyrThrPro LysValGlyGluTyr AlaThrTyrGluAla
125             130             135             140

ATTCGCAAGGCGTTC CGCGTGTGGGAGAGT GCCACACCACTGCGC TTCCGCGAGGTGCCC     600
IleArgLysAlaPhe ArgValTrpGluSer AlaThrProLeuArg PheArgGluValPro
145             150             155             160

TATGCCTACATCCGT GAGGGCCATGAGAAG CAGGCCGACATCATG ATCTTCTTTGCCGAG     660
TyrAlaTyrIleArg GluGlyHisGluLys GlnAlaAspIleMet IlePhePheAlaGlu
165             170             175             180
```

[Sequence Sheet 6]

Sequence No.: 2 (continued)

GGCTTCCATGGCGAC	AGGACGGCCTTCGAT	GGTGAGGGCGGCTTC	CTGGCCCATGCCTAC	720
GlyPheHisGlyAsp	ArgThrAlaPheAsp	GlyGluGlyGlyPhe	LeuAlaHisAlaTyr	
185	190	195	200	
TTCCCAGGGCCCAAC	ATTGGAGGAGACACC	CACTTTGACTCTGCC	GAGCCTTGGACTGTC	780
PheProGlyProAsn	IleGlyGlyAspThr	HisPheAspSerAla	GluProTrpThrVal	
205	210	215	220	
AGGAATGAGGATCTG	AATGGAAATGACATC	TTCCTGGTGGCTGTG	CACGAGCTGGGCCAT	840
ArgAsnGluAspLeu	AsnGlyAsnAspIle	PheLeuValAlaVal	HisGluLeuGlyHis	
225	230	235	240	
GCCCTGGGGCTCGAG	CATTCCAGTGACCCC	TCGGCCATCATGGCA	CCCTTTTACCAGTGG	900
AlaLeuGlyLeuGlu	HisSerSerAspPro	SerAlaIleMetAla	ProPheTyrGlnTrp	
245	250	255	260	
ATGGACACGGAAAAA	TTTGTGCTTCCCCAT	TATGACCCCCGGGGC	ATCCAGCAACTTTAT	960
MetAspThrGluLys	PheValLeuProHis	TyrAspProArgGly	IleGlnGlnLeuTyr	
265	270	275	280	
GGGGGTAAGCAAGGT	TCCCCACCAAGATGC	CCCCTCAACCCAGGA	CTACCTCCCGGCCTT	1020
GlyGlyLysGlnGly	SerProProArgCys	ProLeuAsnProGly	LeuProProGlyLeu	
285	290	295	300	
CTGTTCCTGATAAAC	CCCAAAAACCCCACC	TATGGGCCCAACATC	TGTGACGGGAACTTT	1080
LeuPheLeuIleAsn	ProLysAsnProThr	TyrGlyProAsnIle	CysAspGlyAsnPhe	
305	310	315	320	
GACACCGTGGCGATG	CTCCGAGGGGAGATG	TTTGACTTCAAGAAG	CGCTGGTTCTGGCGG	1140
AspThrValAlaMet	LeuArgGlyGluMet	PheAspPheLysLys	ArgTrpPheTrpArg	
325	330	335	340	
GTGAGGAATAACCAA	GTGATGGATGGATAC	CCAATGCCCATTGGC	CAGTTCTGGCGGGGC	1200
ValArgAsnAsnGln	ValMetAspGlyTyr	ProMetProIleGly	GlnPheTrpArgGly	
345	350	355	360	
CTGCCTGCGTCCATC	AACACTGCCTACGAG	AGGAAGGATGGCAAA	TTCGTCTTCTTCAAA	1260
LeuProAlaSerIle	AsnThrAlaTyrGlu	ArgLysAspGlyLys	PheValPhePheLys	
365	370	375	380	
GGAGACAAGCATTGG	GTGTTTGATGAGGCG	TCCCTGGAACCTGGC	TACCCCAAGCACATT	1320
GlyAspLysHisTrp	ValPheAspGluAla	SerLeuGluProGly	TyrProLysHisIle	
385	390	395	400	

[Sequence Sheet 7]

Sequence No.: 2 (continued)

AAGGAGCTGGGCCCA GGGCTGCCTACCGAC AAGATTGATGCTGCT CTCTTCTGGATGCCC	1380
LysGluLeuGlyArg GlyLeuProThrAsp LysIleAspAlaAla LeuPheTrpMetPro 405 410 415 420	
AATGGAAAGACCTAC TTCTTCCGTGGAAAC AAGTACTACCGTTTC AACGAAGAGCTCAGG	1440
AsnGlyLysThrTyr PhePheArgGlyAsn LysTyrTyrArgPhe AsnGluGluLeuArg 425 430 435 440	
GCAGTGGATAGCGAG TACCCCAAGAACATC AAAGTCTGGGAAGGG ATCCCTGAGTCTCCC	1500
AlaValAspSerGlu TyrProLysAsnIle LysValTrpGluGly IleProGluSerPro 445 450 455 460	
AGAGGGTCATTCATG GGCAGCGATGAAGTC TTCACTTACTTCTAC AAGGGGAACAAATAC	1560
ArgGlySerPheMet GlySerAspGluVal PheThrTyrPheTyr LysGlyAsnLysTyr 465 470 475 480	
TGGAAATTCAACAAC CAGAAGCTGAAGGTA GAACCGGGCTACCCC AAGTCAGCCCTGAGG	1620
TrpLysPheAsnAsn GlnLysLeuLysVal GluProGlyTyrPro LysSerAlaLeuArg 485 490 495 500	
GACTGGATGGGCTGC CCATCGGGAGGCCGG CCGGATGAGGGGACT GAGGAGGAGACGGAG	1680
AspTrpMetGlyCys ProSerGlyGlyArg ProAspGluGlyThr GluGluGluThrGlu 505 510 515 520	
GTGATCATCATTGAG GTGGACGAGGAGGGC GGCGGGGCGGTGAGC GCGGCTGCCGTGGTG	1740
ValIleIleIleGlu ValAspGluGluGly GlyGlyAlaValSer AlaAlaAlaValVal 525 530 535 540	
CTGCCCGTGCTGCTG CTGCTCCTGGTGCTG GCGGTGGGCCTTGCA GTCTTCTTCTTCAGA	1800
LeuProValLeuLeu LeuLeuLeuValLeu AlaValGlyLeuAla ValPhePhePheArg 545 550 555 560	
CGCCATGGGACCCCC AGGCGACTGCTCTAC TGCCAGCGTTCCCTG CTGGACAAGGTCTGA	1860
ArgHisGlyThrPro ArgArgLeuLeuTyr CysGlnArgSerLeu LeuAspLysVal... 565 570 575 580	
CGCCCATCCCCCGGC CCGCCCACTCCTACC ACAAGGACTTTGCCT CTGAAGGCCAGTGGC	1920
AGCAGGTGGTGGTGG GTGGGCTGCTCCCAT CGTCCCGAGCCCCCT CCCCGCAGCCTCCTT	1980

[Sequence Sheet 8]

Sequence No.: 2 (continued)

GCTTCTCTCTGTCCC CTGGCTGGCCTCCTT CACCCTGACCGCCTC CCTCCCTCCTGCCCC 2040

GGCATTGCATCTTCC CTAGATAGGTCCCCT GAGGGCTGAGTGGGA GGGCGGCCCTTTCCA 2100

GCCTCTGCCCCTCAG GGGAACCCTGTAGCT TTGTGTCTGTCCAGC CCCATCTGAATGTGT 2160

TGGGGGCTCTGCACT TGAAGGCAGGACCCT CAGACCTCGCTGGTA AAGGTCAAATGGGGT 2220

CATCTGCTCCTTTTC CATCCCCTGACATAC CTTAACCTCTGAACT CTGACCTCAGGAGGC 2280

TCTGGGGAACTCCAG CCCTGAAAGCCCCAG GTGTACCCAATTGGC AGCCTCTCACTACTC 2340

TTTCTGGCTAAAAGG AATCTAATCTTGTTG AGGGTAGAGACCCTG AGACAGTGTGAGGGG 2400

GTGGGGACTGCCAAG CCACCCTAAGACCTT GGGAGGAAAACTCAG AGAGGGTCTTCGTTG 2460

CTCAGTCAGTCAAGT TCCTCGGAGATCTTC CTCTGCCTCACCTAC CCCAGGGAACTTCCA 2520

AGGAAGGAGCCTGAG CCACTGGGGACTAAG TGGGCAGAAGAAACC CTTGGCAGCCCTGTG 2580

CCTCTCGAATGTTAG CCTTGGATGGGGCTT TCACAGTTAGAAGAG CTGAAACCAGGGGTG 2640

[Sequence Sheet 9]

Sequence No.: 2 (continued)

CACCTGTCAGGTAGG GTGGGGCCCGGTGGGA GAGGCCCGGGTCAGA GCCCTGGGGGTGAGC 2700

CTTAAGGCCACAGAG AAAGAACCTTGCCCA AACTCAGGCAGCTGG GGCTGAGGCCCAAAG 2760

GCAGAACAGCCAGAG GGGGCAGGAGGGGAC CAAAAAGGAAAATGA GGACGTGCAGCAGCA 2820

TTGGAAGGCTGGGGC CCGGCAGCCAGGTTA AAGCTAACAGGGGGC CATCAGGGTGGGCTT 2880

GTGGAGCTCTCAGGA AGGGCCCTGAGGAAG GCACACTTGCTCCTG TTGGTCCCTGTCCTT 2940

GCTGCCCAGGCAGGG TGGAGGGGAAGGGTA GGGCAGCCAGAGAAA GGAGCAGAGAAGGCA 3000

CACAAACGAGGAATG AGGGGCTTCACGAGA GGCCACAGGGCCTGG CTGGCCACGCTGTCC 3060

CGGCCTGCTCACCAT CTCAGTGAGGGACAG GAGCTGGGGCTGCTT AGGCTGGGTCCACGC 3120

TTCCCTGGTGCCAGC ACCCCTCAAGCCTGT CTCACCAGTGGCCTG CCCTCTCGCTCCCCC 3180

ACCCAGCCCACCCAT TGAAGTCTCCTTGGG TCCCAAAGGTGGGCA TGGTACCGGGGACTT 3240

GGGAGAGTGAGACCC AGTGGAGGGAGCAAG AGGAGAGGGATGTGG GGGGGTGGGGCACGG 3300

GTAGGGGAAATGGGG TGAACGGTGCTGGCA GTTCGGCTAGATTTC TGTCTTGTTTGTTTT 3360

TTTGTTTTGTTTAAT GTATATTTTTATTAT AATTATTATATAT

[Sequence Sheet 10]

Sequence No.: 3

Length of sequence: 7

Type of sequence: Amino acid

Topology: Linear

Class of sequence: Peptide

Fragment type: Intermediate fragment

Sequence

Pro Arg Cys Gly Val Pro Asp

1 5

[Sequence Sheet 11]

Sequence No.: 4

Length of sequence: 9

Type of sequence: Amino acid

Topology: Linear

Class of sequence: Peptide

Fragment type: Intermediate fragment

Sequence

Gly Asp Ala His Phe Asp Asp Asp Glu

1 5

[Sequence Sheet 12]

Sequence No.: 5

Length of sequence: 20

Type of sequence: Nucleic acid

Number of chain: Double strand

Topology: Linear

Class of sequence: Other nucleic acid, synthetic DNA

Sequence

CC(C/A)(C/A)G(G/A/C)TG(T/C)(C/G)G(G/A/C)(G/A)(A/T)(G/C/T)CC(T/A)GA

[Sequence Sheet 13]

Sequence No.: 6

Length of sequence: 25

Type of sequence: Nucleic acid

Number of chain: Double strand

Topology: Linear

Class of sequence: Other nucleic acid, synthetic DNA

Sequence

(T/C)TC(G/A)T(G/C)(G/A/C)TC(G/A)TC(G/A)AA(G/A)TG(G/A)(G/A)(C/A/T)(G/A)TC(T/C)

[Sequence Sheet 14]

Sequence No.: 7

Length of sequence: 27

Type of sequence: Amino acid

Topology: Linear

Class of sequence: Peptide

Fragment type: Intermediate fragment

Sequence

Gly Gly Gly Ala Val Ser Ala Ala Ala Val
1 5 10
Val Leu Pro Val Leu Leu Leu Leu Leu Val
15 20
Leu Ala Val Gly Leu Ala Val Phe Phe Phe
25

[Sequence Sheet 15]

Sequence No.: 8

Length of sequence: 14

Type of sequence: Amino acid

Topology: Linear

Class of sequence: Peptide

Sequence

Arg Glu Val Pro Tyr Ala Tyr Ile Arg Glu
1 5 10
Gly His Glu Lys

[Sequence Sheet 16]

Sequence No.: 9

Length of sequence: 14

Type of sequence: Amino acid

Topology: Linear

Class of sequence: Peptide

Sequence

```
Asp  Gly  Asn  Phe  Asp  Thr  Val  Ala  Met  Leu
 1                   5                        10
Arg  Gly  Glu  Met
```

[Sequence Sheet 17]

Sequence No.: 10

Length of sequence: 15

Type of sequence: Amino acid

Topology: Linear

Class of sequence: Peptide

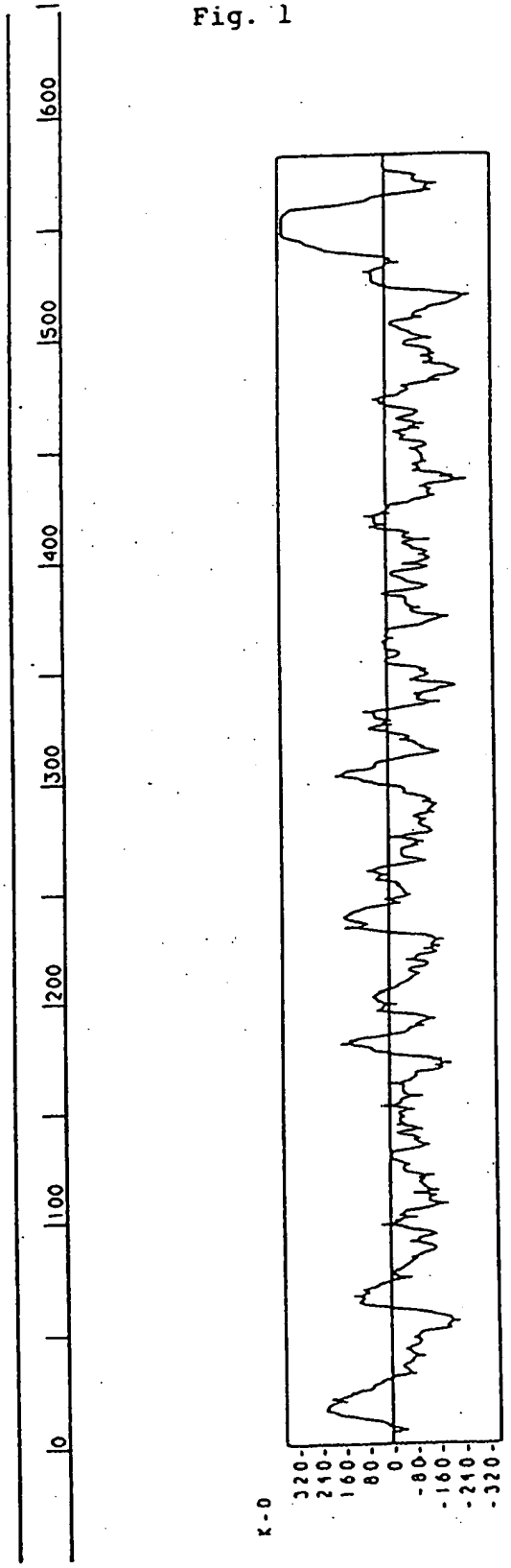
Sequence

```
Pro  Lys  Ser  Ala  Leu  Arg  Asp  Trp  Met  Gly
 1                   5                        10
Cys  Pro  Ser  Gly  Gly
                    15
```

Claims

1. A protein having the amino acid sequence shown in Sequence Sheet sequence number 1.
2. A DNA having the nucleotide sequence shown in Sequence Sheet sequence number 2 which encodes a protein having the amino acid sequence shown in Sequence Sheet sequence number 1.
3. A plasmid containing a DNA having the nucleotide sequence shown in Sequence Sheet sequence number 2, and expressing the protein shown in Sequence Sheet sequence number 1.
4. A host cell harbouring a plasmid containing a DNA having the nucleotide sequence shown in Sequence Sheet sequence number 2, and expressing the protein shown in Sequence Sheet sequence number 1.
5. Monoclonal antibodies which peculiarly recognize a protein having the amino acid sequence shown in Sequence Sheet sequence number 1.

Fig. 1



```
MMP-11          MAPAAWLRSA AARALLPPML LLLLQPPPL- ---------- ------LARA   33
MMP-1           MHS------- ------FPPL LLLLFWGVVS HSFPATLETQ EQDVDLVQKY   37
MMP-8           MFSLKTL--- -------PFL LLLHVQISKA FPVSSKEKNT KTVQD----Y   36
MMP-10          MMHL------ -------AFL VLLCLPVCSA YPLSGAAKEE DSNKDLAQQY   37
MMP-3           MKSL------ -------PIL LLLCVAVCSA YPLDGAARGE DTSMNLVQKY   37
MMP-9           MSLWQP---- ---LVLVLLV LGCCFAAPRQ RQSTLVLFPG DLRTNLTDRQ   43
MMP-2           ---------- ---------- ------AP-- --SPIIKFPG DVAPK-TDKE   19
MMP-7           MR-------- ---LTVLCAV CLLPGSLALP LPQEAGGMSE ------LQWE   33
MT-MMP          MSPAP----- ------RPSR CLLLPLLTLG TALASLGSAQ SSSFS-PEAW   38

Consensus       M..------- -------.P.L LLL..----- ---------- ----------   50


MMP-11          LPPDVHHL-- ---HAERR-G PQPWHAALPS SP---APAPA TQEAPRPASS   74
MMP-1           L-EKYYNLKN DGRQVEKRRN SGPVVEKLKQ MQEFFGLKVT GKPDAETLKV   86
MMP-8           L-EKFYQLPS NQYQSTRKNG TNVIVEKLKE MQRFFGLNVT GKPNEETLDM   85
MMP-10          L-EKYYNLEK DVKQFRRK-D SNLIVKKIQG MQKFLGLEVT GKLDTDTLEV   85
MMP-3           L-ENYYDLKK DVKQFVRRKD SGPVVKKIRE MQKFLGLEVT GKLDSDTLEV   86
MMP-9           LAEEYLYRYG YTRVAEMRGE SKSLGPALLL LQKQLSLPET GELDSATLKA   93
MMP-2           LAVQYLNTF- YGCPKE-SCN LFVLKDTLKK MQKFFGLPQT GDLDQNTIET   67
MMP-7           QAQDYLKRF- YLYDSETK-N ANSLEAKLKE MQKFFGLPIT GMLNSRVIEI   81
MT-MMP          L-QQYGYLPP GDLRTHTQRS PQSLSAAIAA MQKFYGLQVT GKADADTMKA   87

Consensus       L-E.Y..L.. .....E.... ......KL.. MQKF.GL.VT GKLD..TL..  100
```

Fig. 2A

```
MMP·11          LRPPRCGVPD ·PSDGLSARN RQKRFVLSGG RWEKTDLTYR ILRFPWQLVQ   123
MMP·1           MKQPRCGVPD ·VAQ·FVL·· ····TE·GNP RWEQTHLTYR IENYTPDLPR   127
MMP·8           MKKPRCGVPD ·SGG·FML·· ····TP·GNP KWERTNLTYR IRNYTPQLSE   126
MMP·10          MRKPRCGVPD ·VGH·FSS·· ····FP·GMP KWRKTHLTYR IVNYTPDLPR   126
MMP·3           MRKPRCGVPD ·VGH·FRT·· ····FP·GIP KWRKTHLTYR IVNYTPDLPK   127
MMP·9           MRTPRCGVPD ·LGR·FQT·· ····FE·GDL KWHHHNITYW IQNYSEDLPR   134
MMP·2           MRKPRCGNPD ·VAN·YNF·· ····FP·RKP KWDKNQITYR IIGYTPDLDP   108
MMP·7           MQKPRCGVPD ·VAE·YSL·· ····FP·NSP KWTSKVVTYR IVSYTRDLPH   122
MT·MMP          MRRPRCGVPD KFGAEIKANV RRKRYAIQGL KWQHNEITFC IQNYTPKVGE   137

Consensus       MRKPRCGVPD ·VG.·F.··· ····FP·G.P KW..T.LTYR I.NYTPDLP.   150


MMP·11          EQVRQTMAEA LKVWSDVTPL TFTEV····· ···HEGRADI MIDFARYWDG   165
MMP·1           ADVDHAIEKA FQLWSNVTPL TFTKV····· ···SEGQADI MISFVRGDHR   169
MMP·8           AEVERAIKDA FELWSVASPL IFTRI····· ···SQGEADI NIAFYQRDHG   168
MMP·10          DAVDSAIEKA LKVWEEVTPL TFSRL····· ···YEGEADI MISFAVKEHG   168
MMP·3           DAVDSAVEKA LKVWEEVTPL TFSRL····· ···YEGEADI MISFAVREHG   169
MMP·9           AVIDDAFARA FALWSAVTPL TFTRV····· ···YSRDADI VIQFGVAEHG   176
MMP·2           ETVDDAFARA FQVWSDVTPL RFSRI····· ···HDGEADI MINFGRWEHG   150
MMP·7           ITVDRLVSKA LNMWGKEIPL HFRKV····· ···VWGTADI MIGFARGAHG   164
MT·MMP          YATYEAIRKA FRVWESATPL RFREVPYAYI REGHEKQADI MIFFAEGFHG   187

Consensus       ..VD.A..KA F.VWS.VTPL TF.RV····· ···.EG.ADI MI.FA...HG   200
```

Fig. 2B

MMP·11	DDLPFDGPGG	ILAHAFFPKT	HREGDVHFDY	DETWTIGDDQ	GTD·····	208
MMP·1	DNSPFDGPGG	NLAHAFQPGP	GIGGDAHFDE	HERWTNNFT·	EYN·····	211
MMP·8	DNSPFDGPNG	ILAHAFQPGQ	GIGGDAHFDA	EETWTNTSA·	NYN·····	210
MMP·10	DFYSFDGPGH	SLAHAYPPGP	GLYGDIHFDD	DEKWTEDAS·	GTN·····	210
MMP·3	DFYPFDGPGN	VLAHAYAPGP	GINGDAHFDD	DEQWTKDTT·	GTN·····	211
MMP·9	DGYPFDGKDG	LLAHAFPPGP	GIQGDAHFDD	DELWSLGKG·	VVVPTRFGNA	225
MMP·2	DGYPFDGKDG	LLAHAFAPGT	GVGGDSHFDD	DELWTLGEG·	QVVRVKYGNA	199
MMP·7	DSYPFDGPGN	TLAHAFAPGT	GLGGDAHFDE	DERWTDGSSL	GIN·····	207
MT·MMP	DRTAFDGEGG	FLAHAYFPGP	NIGGDTHFDS	AEPWTVRNE·	DLN·····	229
Consensus	D.YPFDGPGG	.LAHAF.PGP	GIGGDAHFD.	DE.WT·····	..N·····	250

MMP·11	·····	·····	·····	·····	·····	208
MMP·1	·····	·····	·····	·····	·····	211
MMP·8	·····	·····	·····	·····	·····	210
MMP·10	·····	·····	·····	·····	·····	210
MMP·3	·····	·····	·····	·····	·····	211
MMP·9	DGAACHFPFI	FEGRSYSACT	TDGRSDGLPW	CSTTANYDTD	DRFGFCPSER	275
MMP·2	DGEYCKFPFL	FNGKEYNSCT	DTGRSDGFLW	CSTTYNFEKD	GKYGFCPHEA	249
MMP·7	·····	·····	·····	·····	·····	207
MT·MMP	·····	·····	·····	·····	·····	229
Consensus	·····	·····	·····	·····	·····	300

Fig. 2C

MMP·11	·····	·····	·····	·····	·····	208
MMP·1	·····	·····	·····	·····	·····	211
MMP·8	·····	·····	·····	·····	·····	210
MMP·10	·····	·····	·····	·····	·····	210
MMP·3	·····	·····	·····	·····	·····	211
MMP·9	LYTRDGNADG	KPCQFPFIFQ	GQSYSACTTD	GRSDGYRWCA	TTANYDRDKL	325
MMP·2	LFTMGGNAEG	QPCKFPFRFQ	GTSYDSCTTE	GRTDGYRWCG	TTEDYDRDKK	299
MMP·7	·····	·····	·····	·····	·····	207
MT·MMP	·····	·····	·····	·····	·····	229
Consensus	·····	·····	·····	·····	·····	350

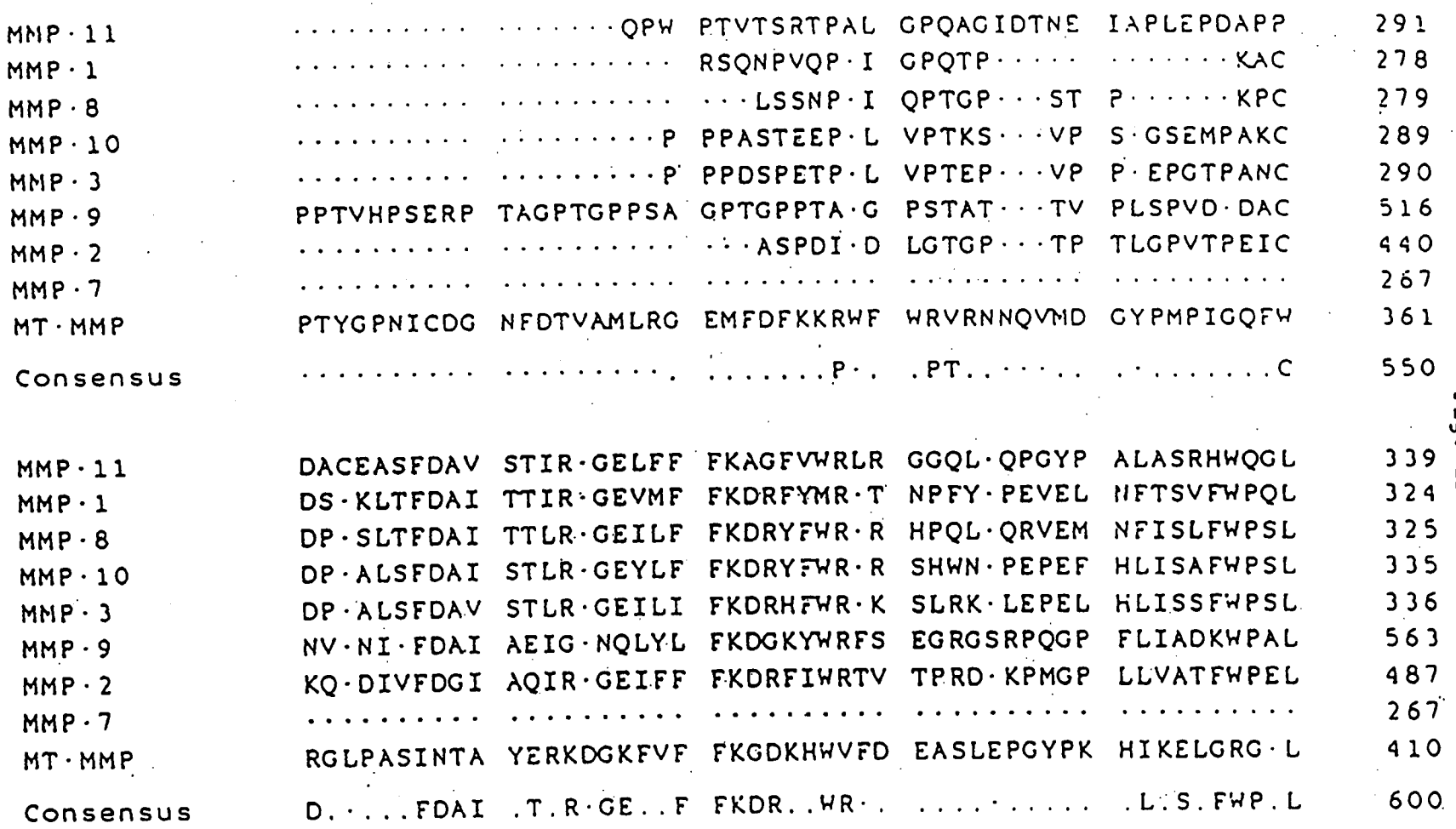
MMP·11	·····	·····	·····	·····	·····	208
MMP·1	·····	·····	·····	·····	·····	211
MMP·8	·····	·····	·····	·····	·····	210
MMP·10	·····	·····	·····	·····	·····	210
MMP·3	·····	·····	·····	·····	·····	211
MMP·9	FGFCPTRADS	TVMGGNSAGE	LCVFPFTFLG	KEYSTCTSEG	RGDGRLWCAT	375
MMP·2	YGFCPETAMS	TVGG·NSEGA	PCVFPFTFLG	NKYESCTSAG	RSDGKMWCAT	348
MMP·7	·····	·····	·····	·····	·····	207
MT·MMP	·····	·····	·····	·····	·····	229
Consensus	·····	·····	·····	·····	·····	400

Fig. 2D

```
MMP·11          ··········  ·········L  LQVAA·HEFG  HVLGLQHTTA  AKALMSAFY·   237
MMP·1           ··········  ·········L  HRVAA·HELG  HSLGLSHSTD  IGALMYPSY·   240
MMP·8           ··········  ·········L  FLVAA·HEFG  HSLGLAHSSD  PGALMYPNY·   239
MMP·10          ··········  ·········L  FLVAA·HELG  HSLGLFHSAN  TEALMYPLYN   240
MMP·3           ··········  ·········L  FLVAA·HEIG  HSLGLFHSAN  TEALMYPLYH   241
MMP·9           TSNFDSDKKW  GFCPDQGYSL  FLVAA·HEFG  HALGLDHSSV  PEALMYPMY·   423
MMP·2           TANYDDDRKW  GFCPDQGYSL  FLVAA·HEFG  HAMGLEHSQD  PGALMAPIY·   396
MMP·7           ··········  ··········  FLYAATHELG  HSLGMGHSSD  PNAVMYPTY·   236
MT·MMP          ··········  ·····GNDI  FLVAV·HELG  HALGLEHSSD  PSAIMAPFY·   261
Consensus       ··········  ·········L  FLVAA·HE.G  HSLGL.HS.D  P.ALMYP.Y·   450

MMP·11          TF··RYPLSL  SPDDCRGVQH  LYG·····  ··········  ··········   258
MMP·1           TF··SGDVQL  AQDDIDGIQA  IYG·····  ··········  ··········   261
MMP·8           AFRETSNYSL  PQDDIDGIQA  IYG·····  ··········  ··········   262
MMP·10          SFTELAQFRL  SQDDVNGIQS  LYG·····  ··········  ··········   263
MMP·3           SLTDLTRFRL  SQDDINGIQS  LYG·····  ··········  ··········   264
MMP·9           RF··TEGPPL  HKDDVNGIRH  LYGPRPEPEP  RPPTTTTPQP  TAPPTVCPTG   471
MMP·2           TY··TKNFRL  SQDDIKGIQE  LYG·····  ··········  ··········   417
MMP·7           GNGDPQNFKL  SQDDIKGIQK  LYGKRSNSRK  K·········  ··········   267
MT·MMP          QWMDTEKFVL  PHYDPRGIQQ  LYGGKQQSPP  RCPLNPGLPP  GLLFLINPKN   311
Consensus       .F.....F.L  SQDDI.GIQ.  LYG·····  ··········  ··········   500
```

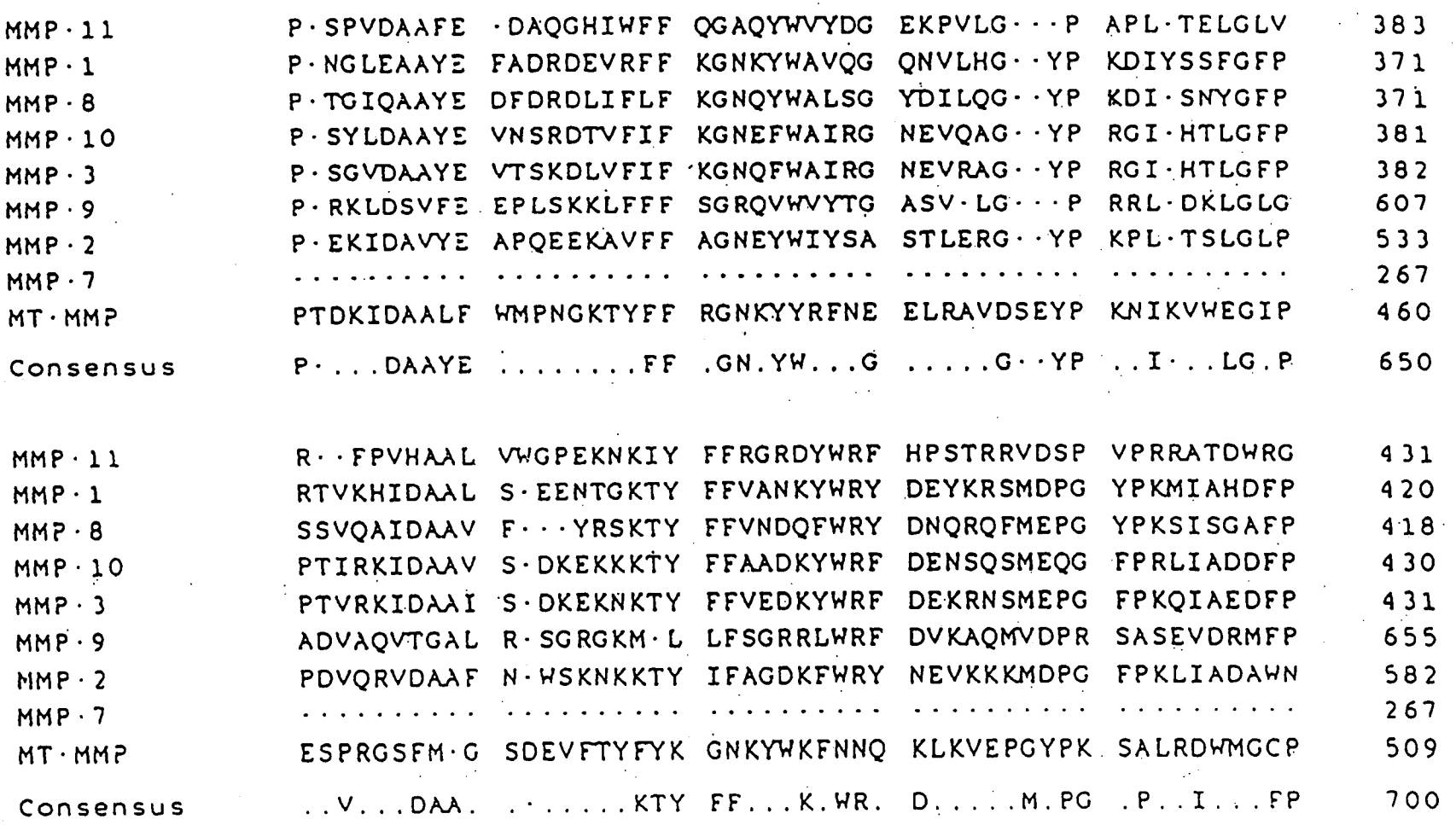
Fig. 2E



MMP·11	.....	.....QPW	PTVTSRTPAL	GPQAGIDTNE	IAPLEPDAPP	291
MMP·1	.....	.....	RSQNPVQP·I	GPQTP·····	.....KAC	278
MMP·8	.....	.....	···LSSNP·I	QPTGP···ST	P·····KPC	279
MMP·10	.....	.....P	PPASTEEP·L	VPTKS···VP	S·GSEMPAKC	289
MMP·3	.....	.....P	PPDSPETP·L	VPTEP···VP	P·EPGTPANC	290
MMP·9	PPTVHPSERP	TAGPTGPPSA	GPTGPPTA·G	PSTAT···TV	PLSPVD·DAC	516
MMP·2	.....	.....	···ASPDI·D	LGTGP···TP	TLGPVTPEIC	440
MMP·7	.....	.....	.....	.....	.....	267
MT·MMP	PTYGPNICDG	NFDTVAMLRG	EMFDFKKRWF	WRVRNNQVMD	GYPMPIGQFW	361
Consensus	.....	.....	.....P·.	.PT.....	C	550

MMP·11	DACEASFDAV	STIR·GELFF	FKAGFVWRLR	GGQL·QPGYP	ALASRHWQGL	339
MMP·1	DS·KLTFDAI	TTIR·GEVMF	FKDRFYMR·T	NPFY·PEVEL	NFTSVFWPQL	324
MMP·8	DP·SLTFDAI	TTLR·GEILF	FKDRYFWR·R	HPQL·QRVEM	NFISLFWPSL	325
MMP·10	DP·ALSFDAI	STLR·GEYLF	FKDRYFWR·R	SHWN·PEPEF	HLISAFWPSL	335
MMP·3	DP·ALSFDAV	STLR·GEILI	FKDRHFWR·K	SLRK·LEPEL	HLISSFWPSL	336
MMP·9	NV·NI·FDAI	AEIG·NQLYL	FKDGKYWRFS	EGRGSRPQGP	FLIADKWPAL	563
MMP·2	KQ·DIVFDGI	AQIR·GEIFF	FKDRFIWRTV	TPRD·KPMGP	LLVATFWPEL	487
MMP·7	.....	.....	.....	.....	.....	267
MT·MMP	RGLPASINTA	YERKDGKFVF	FKGDKHWVFD	EASLEPGYPK	HIKELGRG·L	410
Consensus	D.....FDAI	.T.R·GE..F	FKDR..WR·.	.....L.S.FWP.L		600

Fig. 2F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MMP·1 P·NGLEAAYE FADRDEVRFF KGNKYWAVQG QNVLHG··YP KDIYSSFGFP 371
MMP·8 P·TGIQAAYE DFDRDLIFLF KGNQYWALSG YDILQG··YP KDI·SNYGFP 371
MMP·10 P·SYLDAAYE VNSRDTVFIF KGNEFWAIRG NEVQAG··YP RGI·HTLGFP 381
MMP·3 P·SGVDAAYE VTSKDLVFIF KGNQFWAIRG NEVRAG··YP RGI·HTLGFP 382
MMP·9 P·RKLDSVFE EPLSKKLFFF SGRQVWVYTG ASV·LG···P RRL·DKLGLG 607
MMP·2 P·EKIDAVYE APQEEKAVFF AGNEYWIYSA STLERG··YP KPL·TSLGLP 533
MMP·7 267
MT·MMP PTDKIDAALF WMPNGKTYFF RGNKYYRFNE ELRAVDSEYP KNIKVWEGIP 460
Consensus P·...DAAYE .....FF .GN.YW...G .....G··YP ..I·..LG.P 650
MMP·11 R··FPVHAAL VWGPEKNKIY FFRGRDYWRF HPSTRRVDSP VPRRATDWRG 431
MMP·1 RTVKHIDAAL S·EENTGKTY FFVANKYWRY DEYKRSMDPG YPKMIAHDFP 420
MMP·8 SSVQAIDAAV F···YRSKTY FFVNDQFWRY DNQRQFMEPG YPKSISGAFP 418
MMP·10 PTIRKIDAAV S·DKEKKKTY FFAADKYWRF DENSQSMEQG FPRLIADDFP 430
MMP·3 PTVRKIDAAI S·DKEKNKTY FFVEDKYWRF DEKRNSMEPG FPKQIAEDFP 431
MMP·9 ADVAQVTGAL R·SGRGKM·L LFSGRRLWRF DVKAQMVDPR SASEVDRMFP 655
MMP·2 PDVQRVDAAF N·WSKNKKTY IFAGDKFWRY NEVKKKMDPG FPKLIADAWN 582
MMP·7 267
MT·MMP ESPRGSFM·G SDEVFTYFYK GNKYWKFNNQ KLKVEPGYPK SALRDWMGCP 509
Consensus ..V...DAA. .·.....KTY FF...K.WR. D.....M.PG .P..I...FP 700

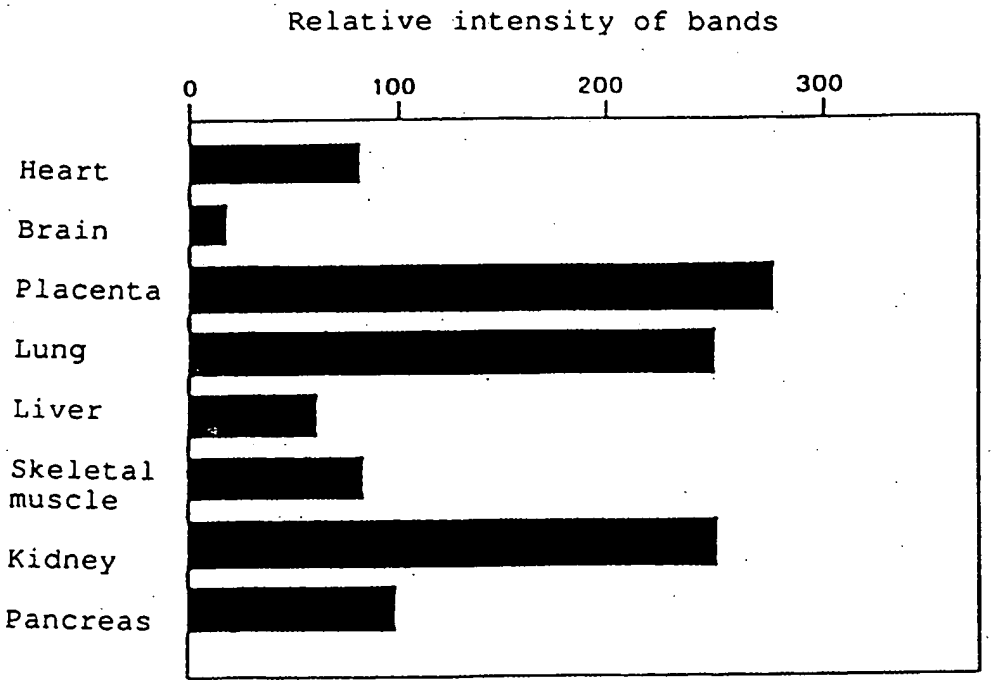
Fig. 2G

```
MMP·11          VPSE··IDAA FQDADGYAYF LRGRLYWKFD PVKVKALEGF PRLV·····      473
MMP·1           GIGH··KVDA V··FMKDGFF ·····YF·FH GTRQYKFDPK TKRILTLQ··      458
MMP·8           GIES··KVDA V··FQQEHFF ·····HV·FS GPRYYAFDLI AQRVTRVA··      456
MMP·10          GVEP··KVDA V··LQAFGFF ····:YF·FS GSSQFEFDPN ARMVTHIL··      468
MMP·3           GIDS··KIDA V··FEEFGFF ·····YF·FT GSSQLEFDPN AKKVTHTL··      469
MMP·9           GVPL··DTHD VFQYREKAYF ···CQDR·FY WRVSSRSELN QVDQVGYV··      697
MMP·2           AIPD··NLDA VVDLQGGG·· ·····HS·YF FKGAYYLKLE N·QSLKSVKF      621
MMP·7           ·········· ·········· ·········· ·········· ··········      267
MT·MMP          SGGRPDEGTE EETEVIIIEV DEEGGGAVSA AAVVLPVLLL LLVLAVGLAV      559
Consensus       G.....DA V·.....F ·····F. ·····  ·····  ·····      750

MMP·11          ···GPD·FFG CAE·····PA NTFLX·····  ·····  ·····      489
MMP·1           ···KANSWFN CR·····KN ·····  ·····  ·····  ·····      469
MMP·8           ···RGNKWLN CRY·····GX ·····  ·····  ·····  ·····      468
MMP·10          ···KSNSWLH C·····  ·····  ·····  ·····  ·····      476
MMP·3           ···KSNSWLN C·····  ·····  ·····  ·····  ·····      477
MMP·9           ···TYD·ILQ CPE·····DX ·····  ·····  ·····  ·····      708
MMP·2           GSIKSD·WLG C·····  ·····  ·····  ·····  ·····      631
MMP·7           ·····  ·····  ·····  ·····  ·····  ·····      267
MT·MMP          FFFRRHGTPR RLLYCQRSLL DKV·····  ·····  ·····  ·····      582
Consensus       ·····.WL. C·····  ·····  ·····  ·····  ·····      796
```

Fig. 2H

Fig. 3


Relative intensity of bands
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100
200
300
Heart
Brain
Placenta
Lung
Liver
Skeletal muscle
Kidney
Pancreas

Fig. 4

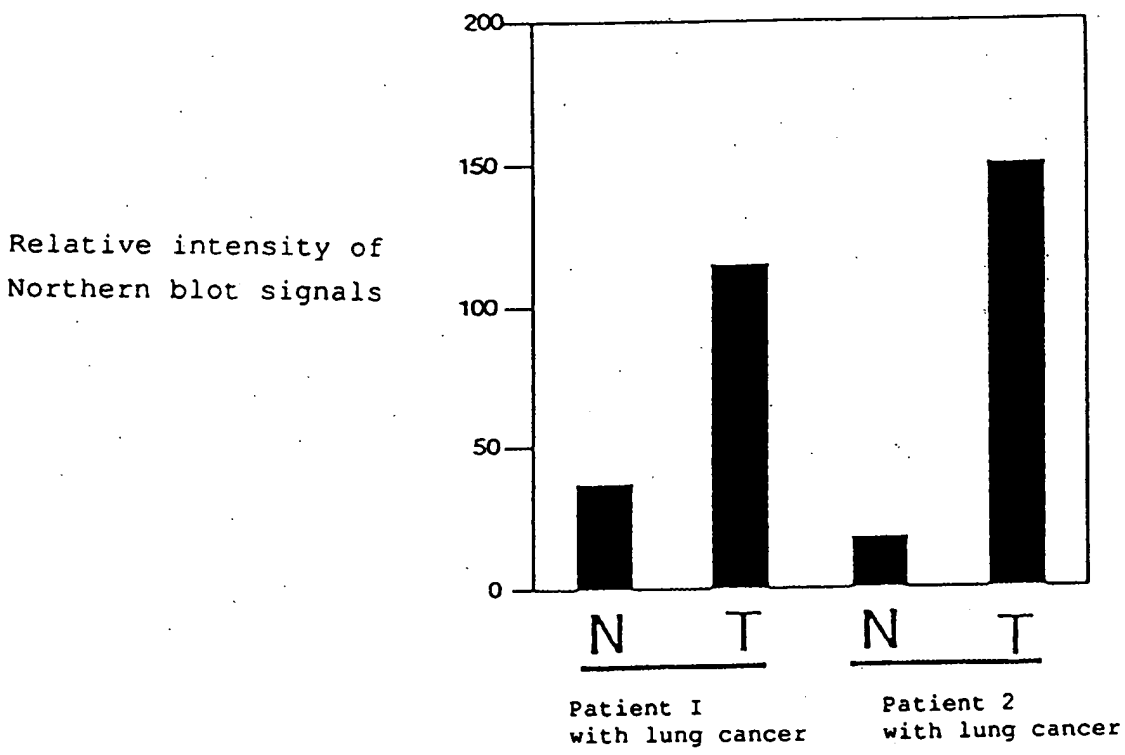

Relative intensity of
Northern blot signals
200
150
100
50
0
N
T
N
T
Patient I
with lung cancer
Patient 2
with lung cancer

Fig. 5

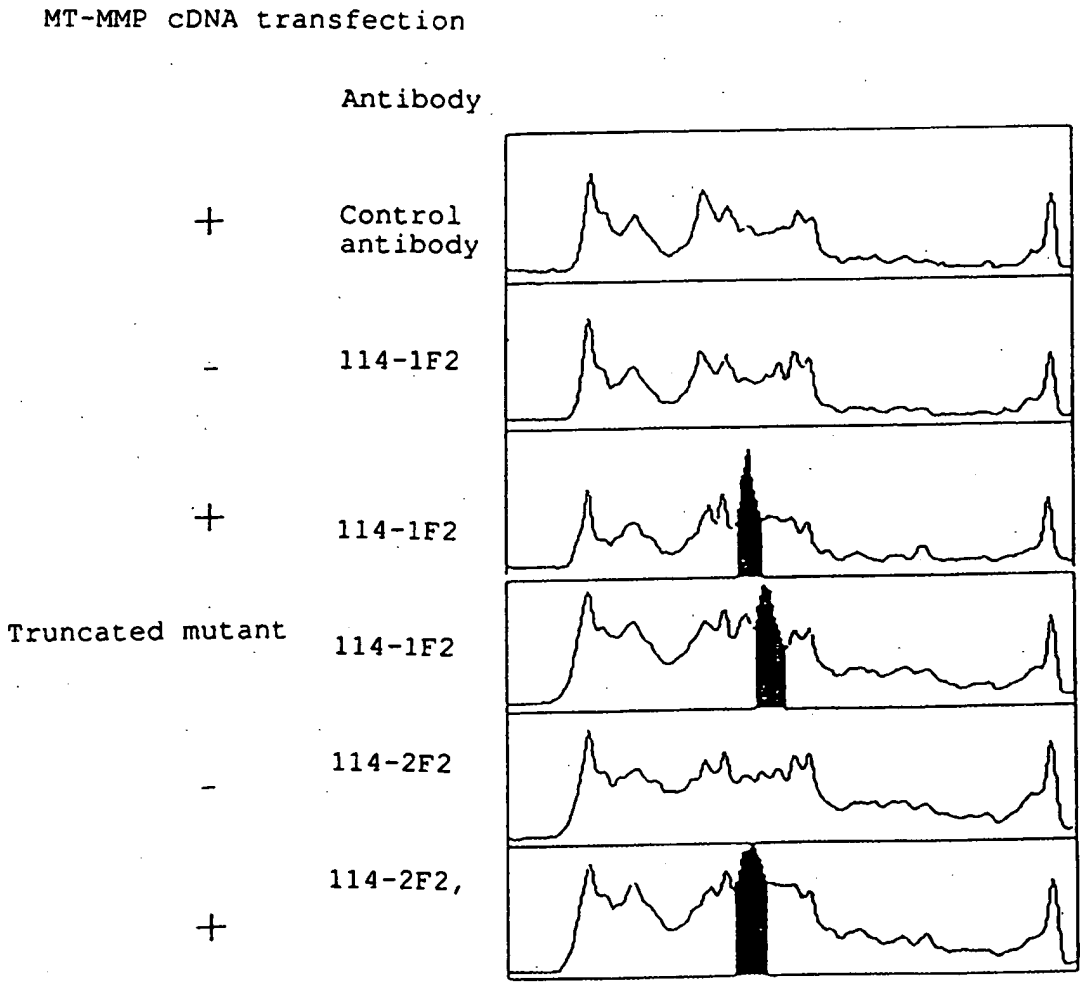

MT-MMP cDNA transfection
Antibody
+
Control antibody
-
114-1F2
+
114-1F2
Truncated mutant
114-1F2
-
114-2F2
114-2F2,
+

Fig. 6

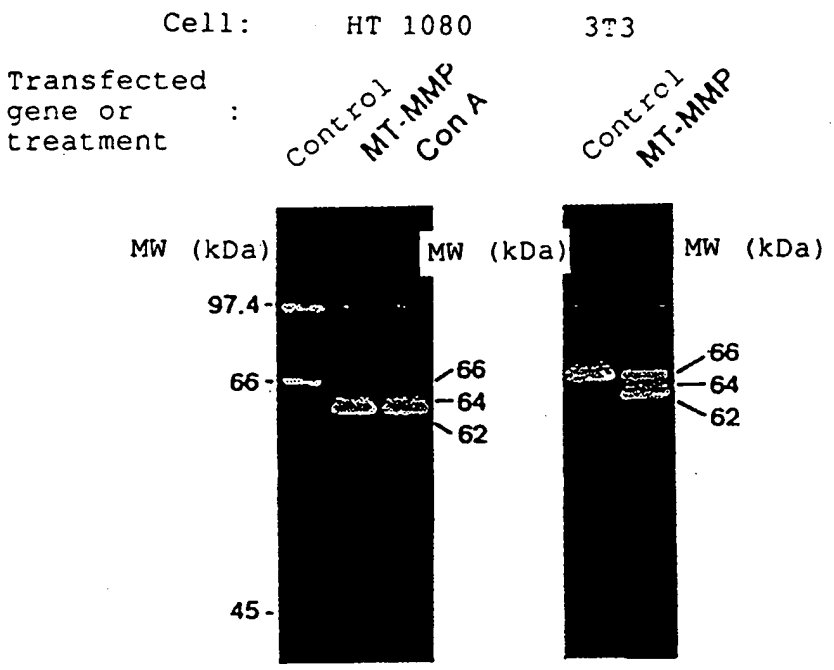

Cell:
HT 1080
3T3
Transfected gene or treatment :
Control
MT-MMP
Con A
Control
MT-MMP
MW (kDa)
MW (kDa)
MW (kDa)
97.4
66
45
66
64
62
66
64
62

Fig. 7

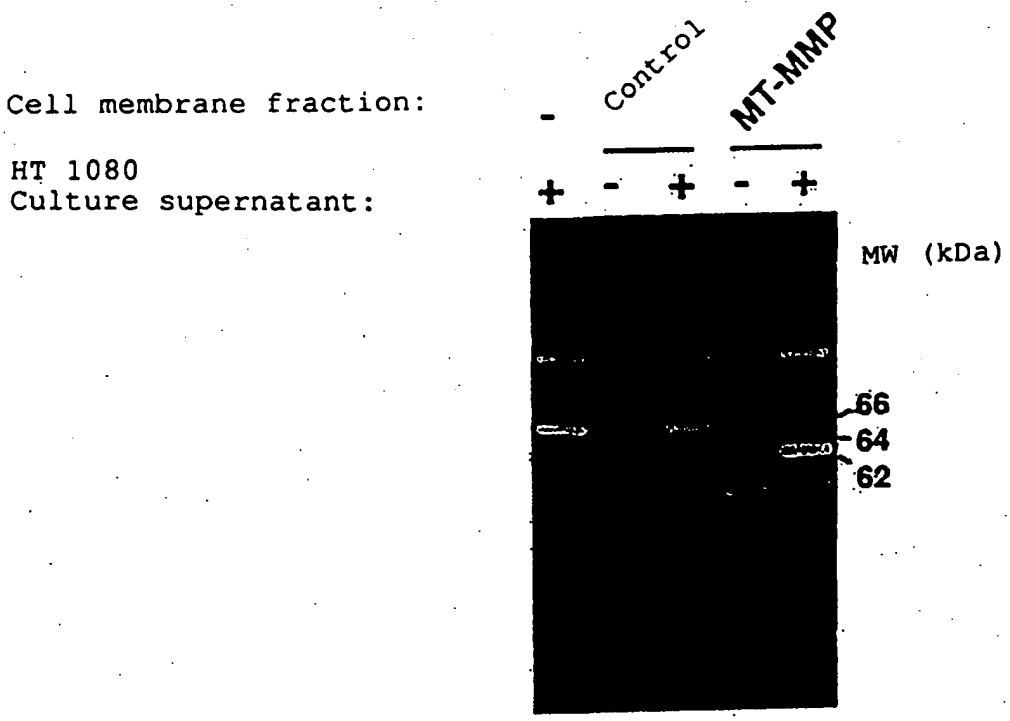
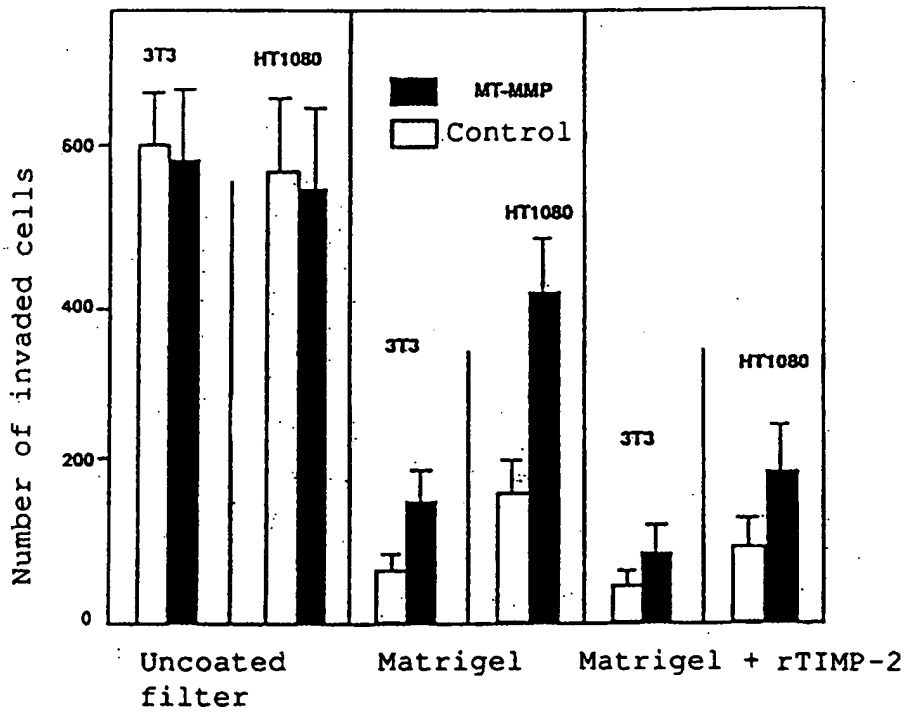

Cell membrane fraction:
HT 1080
Culture supernatant:
-
Control
MT-MMP
+
-
+
-
+
MW (kDa)
66
64
62

Fig. 8


3T3
HT1080
MT-MMP
Control
HT1080
3T3
3T3
HT1080
Number of invaded cells
600
400
200
0
Uncoated filter
Matrigel
Matrigel + rTIMP-2

INTERNATIONAL SEARCH REPORT

International application No.
PCT/JP94/02009

A. CLASSIFICATION OF SUBJECT MATTER

Int. Cl^{6} C12N9/64

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

Int. Cl^{5} C12N9/50-9/64

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

BIOSIS PREWIEWS, WPI, CAS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
PX	Nature, Vol. 370, No. 6484, July 7, 1994 (07. 07. 94), Hiroshi Sato et al. "A matrix metalloproteinase expressed on the surface of invasive tumour cells" P. 61-65	1-5
A	Journal of Cancer Research Clinical Oncology, Vol. 117, No. 2, (1991), M. Siadat Pajour et al. "Expression of metalloproteinase genes in human prostate cancer" P. 144-150	1-5

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
January 20, 1995 (20. 01. 95)	February 7, 1995 (07. 02. 95)
Name and mailing address of the ISA/ Japanese Patent Office	Authorized officer
Facsimile No.	Telephone No.

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